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# ETIOLOGY *and* TREATMENT *of* LEUKEMIA

Proceedings of the First Louisiana Cancer Conference

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# PREFACE

The interest and color which sometimes illuminate recorded thought are often lost in the usual encyclopedic approach of scientific communication. The information included within these covers is set down with an effort to retain some of the spontaneity and flavor of its original presentation. A short space of time was required for the presentation of the proceedings from which this volume arose, and the reader should be able to participate vicariously in an even more abbreviated interval. The ideas and exchanges between participants who are actively obtaining and applying information about leukemia are recorded. This volume represents an arrested instant during a memorable era when results justify hope that current methodology in the field of oncology may indeed lead to control of the disease. The bibliography and summary passages may interest those who desire a slightly more comprehensive survey of etiology and treatment.

The proceedings from which this volume arose were first proposed as part of the professional education program of the American Cancer Society in Louisiana.[332] The Board of Directors made it possible through their active support and allocation of funds for the purpose. Mr. Harry McEnerny, President, Dr. E. T. Krementz and his Committee on Local Arrangements, and Mrs. Marian Simmons, Mr. Henry Houser, and Miss Brent Robertson of the Staff arranged for the meeting. The editorial work was done for the most part under exceedingly pleasant circumstances and surroundings at St. Louis University, and the secretarial work was performed by Miss Judy Felker, Mrs. Helen Kane, and Mrs. Esther Smith. Acknowledgment is also due Dr. M. M. Wintrobe, who has been kind enough to review the final chapter. The competence, interest, and animated discussion of the participants are responsible for whatever profitable material is contained in the volume and for the hope that future conferences will have equal value. To them, the Board of Directors and the Staff of the Louisiana Division, American Cancer Society, the editor is deeply indebted.

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*Jerome T. Syverton, MD*

# VIRUSES *and* CANCER*

When Gross[1] recently established the viral etiology of mouse leukemia, the role of viruses in the induction of neoplasms was re-emphasized. Consideration of new findings of this type is best based on a background knowledge of other types of neoplasia now known associated with a virus as causative agent, and this latest characterization of a mammalian virus-induced tumor should not be used to advance again the general virus theory of cancer. That theory already has been responsible for its share of the hopeful enthusiasms and discouraging disappointments of cancer research. The secret of successful research involves as much the asking of proper questions as the seeking of useful answers. Surely, the proper question is not whether *all* tumors are caused by viruses, but *what* tumors are so caused, and how. Rational consideration of our knowledge of viruses and tumors will avoid alike rejection by the virologist of the virus-induced growths as neoplasms and rejection by the oncologist of tumor agents as viruses. Between the two extremes, we can evaluate old accomplishments and plan new investigations.

Both those familiar with the concepts of neoplasia and malignancy and those who feel they know what a virus is find it difficult to provide exclusive definitions As experimental biologists, we are not limited by the static concepts of pathology. The primary phenomenon of interest, neoplastic growth, is a growth of body cells capable of progression on transfer to other appropriate hosts The phenomenon of malignancy, recognized by cellular invasion or infiltration of contiguous tissue and dissemination by cellular metastasis, is a

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reflection of the degree of autonomy attained by the neoplastic cells. It is not necessary that neoplasia and malignancy be referable to the same etiology. Virologists commonly conceive of a virus as an obligate intracellular parasite, that is, an agent capable of inducing its own replication by susceptible cells on which it is utterly dependent and yet to which it is essentially foreign. We recognize a virus by characteristic histo- or cytopathic effect; we know that the *effect is associated with physical replicating particles, 8 to 300 mμ in size,* of nucleoprotein constitution, with antigenic capacity, usually insusceptible to chemotherapeutic and antibiotic agents. The concept of "virus" is fully as complex as that of "neoplasm"; in either case it is much easier to distinguish the normal and abnormal in practice than it is to isolate the essence of difference. The working definitions are designed mostly to differentiate types of abnormality. The oncologist is intent on the separation of hyperplasia from neoplasia; the virologist, on the distinctions among viruses, bacteria, fungi, and rickettsia The transmissible tumors, like the latest instance which is the subject of this conference, demand more subtle decisions, such as whether the inducing agent is responsible for the neoplastic quality of cellular proliferation, and whether the behavior of the inducing agent is consistent with what we should expect of a virus With these questions in mind, it is profitable to examine the tumors for which decisions already have been rendered

AVIAN SARCOMAS

The study of virus-induced avian tumors was initiated in 1910 with the discovery of the Rous sarcoma of chickens and the Fujinami sarcoma of ducks The Rous sarcoma or Chicken Tumor 1, which now is a classic, experimental tumor,[2] originated in a pedigreed stock of domestic fowls[3] Like mammalian transplanted tumors, it could at first be passed only in blood-related hosts, failing in other strains of the same stock By slow adaptation, the host range of the tumor gradually transcended the barriers of stock, breed, and even species Rous's work showed that Chicken Tumor 1 possessed and propagated an acellular agent capable of inducing the de novo origin of other typical neoplasms classifiable as malignant Distinct transmissible agents, viruses, now are known to be concerned in the etiology of many other fowl tumors, including fibrosarcomas, polymorphic sarcomas, round cell sarcomas, ordinary spindle cell sarcomas, osteochondrosarcomas, and endotheliomas As yet, no avian carcinoma has been attributed to viral infection Except for their virus etiology these avian connective tissue tumors are entirely analogous to the wide variety of mammalian sarcomas, and hence have been accepted as typical neoplasms

The viruses originating these transmissible avian tumors not only are responsible for the neoplastic conversion, but also determine the structural and developmental properties of each particular tumor type. As yet, it has not been

possible to establish beyond doubt which cell types of the connective tissue series commonly give rise to virus-induced tumors. The direction of tumor development dictated by the infecting virus may result from infection of different cells by the same or variant forms of virus, or by modulation of cells after infection in response to the influence of host factors. Recent advances in tissue culture, whereby homogeneous cell populations can be propagated from single cells with reasonable regularity,[4] may hasten solutions to these problems. The influence of host factors on the primary cellular phenomenon has been demonstrated dramatically by the work of Duran-Reynals.[5] Virus introduced intravenously into young chicks produced a lethal hemorrhagic disease; injection of older birds or those previously protected with antibody resulted in typical sarcomas.

The properties of Rous virus are interesting but not extraordinary. It is about 48 mμ in size[6]; it is extremely labile in the absence of protein, purified preparations rapidly losing infectivity at 37° C., although they can be stored indefinitely at −70° C. In the presence of protein, Rous virus is remarkably resistant to desiccation, exposure to glycerol, or to irradiation This resistance to irradiation has been interestingly demonstrated by Peacock.[7] Primary sarcomas vanished upon irradiation with doses of 4,000 to 10,000 r; the virus, not inactivated, was resorbed through lymphatic channels, entered the general circulation, and was fixed at the periphery of the irradiated zone, where it induced formation of satellite tumors

The biologic behavior of the Rous agent in no way gives reason for its rejection as a virus The painstaking efforts of Rubin[8] lately have demonstrated that (a) a single particle can initiate tumor development, (b) virus of a single clone can infect ectodermal or mesodermal cells, and (c) successive generations of multiplying sarcoma cells continually release a few virus particles from each cell, so that virus production keeps pace with cell division The problem of earlier sporadic failures to recover virus from sarcomas, which occasioned some doubt of the causal relationship between virus and neoplastic induction, has been resolved. Rubin's results showed that the proportions of infectious and thermally inactivated virus present in sarcoma cell cultures were determined by the rate of cellular multiplication Further, Bryan and his associates[9] found that the amount of virus recoverable from tumors was a function of initiating dose

In spite of the weight of evidence accumulated from such careful studies, a considerable confusion of thought still exists with respect to the role of Rous virus in the production of neoplasms For example, Warburg,[10] writing "On the Origin of Cancer Cells," had this to say

> If the Rous agent is inoculated into the chorion of chick embryos, tumors originate in the course of a few days as rapidly as in the transplantation of cancer cells The tumors formed are not chorion tumors,

> but Rous sarcomas. The Rous agent to which a particle weight of 150 million is ascribed at present is therefore capable of transmitting the morphological properties of the Rous sarcoma; and whatever we call the Rous agent—"hereditary unit," cell fragment, microcell or spore—the transmission of the Rous sarcoma by the Rous agent is, in any case, nothing more than a *transplantation*, and is to be differentiated strictly from the production of a chicken sarcoma by methylcholanthrene, which is a *neoformation* of a tumor from normal body cells and as such takes a long time.

Despite Professor Warburg's misconception of the common meaning of "transplantation," which is the transfer of intact cells from one host to another, the properties of Rous virus cited in his comments are evidence of tumorigenic action on initially normal cells.

## AVIAN LEUKOSES

Avian leukosis represents a complex of malignant neoplastic processes with viral etiology proved[11] by Ellermann and Bang in 1908. In retrospect, it is understandable why this discovery was not given adequate recognition: avian leukosis was considered a "spontaneous" neoplastic disease because it occurred sporadically, was refractory to transmission even by grafting, and seldom yielded a filtrable agent. Since 1917, the small flocks of mixed breeds maintained in earlier practice have been replaced with inbred lines of birds selected by trap nesting for egg production. These superior stocks and enormous incubators resulted in vast numbers of chickens—ideal populations for virus propagation. Veritable epizootics of avian leukosis were the result. Lines of chickens have been evolved with incidences of leukosis limited only by death of the pullets before egg-bearing maturity; mortalities exceed 40 per cent. In these circumstances, the disease is readily transmissible. A sporadic disease which closely simulated human leukemia in its epidemiologic features thus has been converted by selective host breeding into an infectious disease of epizootic manifestation Genetic and endocrine influences play a determining role in the etiology of avian leukosis, with virus as the causative agent

There are two types of avian leukosis. lymphomatosis and erythromyeloblastosis Lymphomatosis originates with cells of the lymphocytic series, and varies principally with respect to the location of the affected primitive cells. The disease commonly infiltrates the viscera to produce visceral lymphomatosis, it can enter nerve trunks to result in neurolymphomatosis, but rarely invades the circulation. Isolated lymphosarcomas represent another manifestation While lymphomatosis is common, erythromyeloblastotic leukosis is rare. The latter condition, which essentially is a highly malignant leukemia of erythroblasts or myeloblasts, has been subjected to intense and rewarding laboratory study

Although many questions yet remain without answers, the systematic approach of Beard and associates[12] already has illuminated broad areas of the problem of erythromyeloblastosis, and it has provided a model for investigation of other neoplasms of known or suspected virus origin.

It appears that lymphomatosis and erythromyeloblastosis are transmitted specifically and independently by different viruses. The virus of erythromyeloblastosis (EMB) has received the bulk of study. It has been visualized by electron micrography and studied by physicochemical means. A single viral particle is about 140 mμ in diameter. Plasma from infected chickens can contain from 60,000 to 2 trillion ($2 \times 10^{12}$) such particles per milliliter; usually more than 60,000 particles are required to initiate infection. Similar to influenza virus, erythromyeloblastosis virus has an intimately associated enzymatic component, in this instance, adenosine and inosine triphosphatase. The virus now can be assayed quantitatively by electron micrographic particle enumeration, infectivity bioassay, or enzyme-activity assay. Although infection is not always accompanied by production of neutralizing antibody, specific hyperimmune serums can be produced in rabbits or chickens. Such serums do not affect enzyme activity. The intimacy of relationship between virus and host cell is emphasized (a) by the neutralization of infectivity by antiserums induced in rabbits, guinea pigs, or mice against either concentrated virus or normal chick tissue and (b) by the presence of Forssman antigen in viral concentrates. The evidence found by Beard and his associates[12] strongly indicates that normal chick and Forssman antigens exist as integral parts of the virus. Since typical antiviral antibodies can be produced experimentally with adequate doses of virus, it is evident that the irregular natural immune response derives from low dosage. Investigators of other neoplastic conditions should note that lack of an antibody response is not sufficient to rule out virus etiology.

### MAMMARY CARCINOMA OF MICE

Since the turn of the century, the mouse mammary tumor probably has been the most popular tool for cancer research. Prior to 1936, many thousands of hours had been invested in the delineation of its biology; it was the prototypical example of an experimental, spontaneous tumor, a true mammalian malignant neoplasm, ideal for investigation. With the development of inbred or homozygous strains of mice,[13] contributed by the outstanding efforts of Strong, Little, and others, the mammary tumor could be transplanted at will. Work with inbred stocks proved the importance of genetic factors in the development of cancer of the breast. In this early period, the investigations of Lathrop and Loeb[14] emphasized the role of hormonal factors. The suggestion in 1933 by Little and Korteweg of the essential action of an extrachromosomal influence, and the culminating demonstration in 1936 by Bittner[15] of a causative, transmissible agent, finally characterized mouse mammary cancer as a virus-induced

neoplasm.[16] The virus has been hedgingly described by such noncommittal terms as "milk influence," "milk factor," and "Bittner agent," but it evinces the properties by which a virus is known, and it can be so called.

Mouse mammary tumor virus is distributed throughout the bodies of mice of strains with a high incidence of mammary cancer. It is readily transmissible to susceptible mice through mothers' milk by nursing, and by milk or extracts of assorted tissues by injection. The precise site of infection is not known, nor is it known whether the overt manifestation of mammary adenocarcinoma results from an actuating effect of the virus or from a provocative influence similar to that exerted by Shope virus in the rabbit papilloma-to-carcinoma sequence. Strains of mice exist with a fair incidence of breast cancer, from which no virus has been isolated.[17] We are, however, reminded by the work with avian neoplasms that inability to recover virus by bioassay of crude extracts is no guarantee of its absence. Mammary tumor virus has not been as well characterized biologically, physically, or immunologically as the other oncogenic viruses, for reasons of obvious difficulty. The latent period of infection alone is a formidable barrier to bioassay, ranging from six to twelve or more months. It is known that the virus is antigenic in heterologous species, but that, curiously, it is not antigenic in mice. This lack of immune response in the susceptible host cannot be attributed to low dosage; detectable antibodies are not produced by hyperimmunization procedures. It is possible that host proteins are even more intimately associated with the virus than is the case with avian erythromyeloblastosis virus The common development of breast cancer in mice, however incomplete our knowledge of it, reflects an essential role for a causative virus, and the inseparable influence and interplay of genetic factors and hormones as well.

## PAPILLOMATOSIS OF RABBITS

A cutaneous papilloma occurs enzootically among wild cottontail rabbits in states bordering the Mississippi River. In 1933, Shope[18] demonstrated that the benign papilloma was virus-induced. Reports by Rous and Beard[19] that the horny growths could progress to frank epidermoid carcinoma raised hopes that a mammalian cancer of viral origin was available for study.

During the twenty years from 1934, in our own laboratory more than 2,000 wild, cottontail, and domestic Dutch belt rabbits with papillomatosis were observed [20] Weekly examination of 600 of these, kept as undisturbed as possible, revealed the natural pattern of tumor growth Papillomatosis was found to progress predictably through three phases proliferative, stationary, and involutionary The proliferative phase begins seven to forty-two days after exposure of injured epidermis to virus, when tiny vesiculoid papules appear The infected epithelial cells multiply into growths ranging in size from small nodules

to enormous verrucous masses and cutaneous horns In three to six months, a stationary phase supervenes in which lesions are quiescent, without evidence of degeneration or further proliferation. This phase commonly is of shorter duration in the experimental host, the domestic rabbit. The growth cycle terminates in an involutionary phase. Either the final failure of the germinal layer to continue growth results in regression and disappearance of the lesion, or renewed proliferative vigor ensues, with replacement of benign by malignant cells and formation of epidermoid carcinoma The progression to carcinoma has been observed in 75 per cent of domestic and 25 per cent of wild rabbits naturally or experimentally infected.

The causative agent of papillomatosis, Shope's virus, is about 44 to 66 mμ in size,[12] and the virus is extremely resistant to deterioration. All of the antigenic and infective properties of the virus are attributable to the elementary particles; no evidence of associated enzymes or host antigens exists Cytotrophism is limited strictly to certain skin cells If virus is inoculated along a long scarification passing across epidermis and mucous membrane, papilloma formation stops abruptly at the beginning of mucous membrane. Virus must reach the epidermal basal cell layer to become effective. The number of particles reaching the critical site determine the incubation period and the number, extent, and confluence of lesions Virus cannot be isolated from growths with low content of virus because virus concentrations sufficient to infect the critical site in the test host are not obtained Such difficulty of isolation with domestic rabbit papillomas led to the postulation of a "masked" state[21] of virus, it seems clear now that insufficient virus was the case.

Infective virus is recoverable from proliferating, quiescent, or even regressing papillomas It has been recovered commonly from these papillomas in cottontails, but rarely from domestic rabbits; it has not been recovered from carcinomas. Apparently unquestionable evidence of a persistent association between virus and cancer was obtained, however, by Kidd and Rous[22] with the V-2 carcinoma, with which successive transplantations over many years regularly induced virus-neutralizing antibodies in recipient hosts The immune response ceased after 50 passages.[23] We know that papilloma formation is a direct result of virus infection, we do not know the role of virus in the neoplastic transformation The papilloma-to-carcinoma sequence suggests three possibilities (1) the ultimate cancer is produced by papilloma virus; (2) a second virus specifically inducing the carcinoma is activated by the antecedent papilloma, (3) papilloma virus may persist in the succeeding carcinoma as an incidental passenger If either of the first and second alternatives is true, virus should be recoverable not only from the primary carcinoma but also from the secondary, pulmonary metastases It is not. The relation between virus and carcinoma remains unknown.

In summary, it can be said that five avian and mammalian tumors, at least three primarily malignant, have been accepted as virus-induced. The complexity of the agents varies from that of rabbit papilloma virus to that of chicken sarcoma virus with its variability in mode of infection, pathogenicity, and cytotrophism by species, race, tissue, and cell. The range of complexity and the properties involved are no stranger than those of other viruses. Oncogenic viruses certainly are responsible for the proliferative origin of the tumors; whether they are always necessarily responsible for the ultimate neoplastic quality of the growths is not decided. The answer seems to vary from a definite "yes" with avian sarcoma and erythromyeloblastosis, to a "maybe" with mouse mammary carcinoma, to a "probably not" with rabbit epithelioma. We may hope that extension of the study of mouse leukemia virus, already so vigorously prosecuted by Gross,[24] Law and associates,[25] and Stewart,[26] will shed further light on the relationship of viruses to cancer.

## DISCUSSION

DR. BURDETTE. Dr. Fox, will you open the discussion of Dr. Syverton's paper?

DR. FOX: Thank you, Dr. Burdette. I have been trying to relate the information presented about rodent disease to human application. Each of us, *of course, does this in his own way, conditioned by his own peculiar experience.* Like Dr Syverton, I am interested in viruses and sometimes labor under the handicap of being called a virologist Also, I am interested in epidemiology, and I wish to say at this point that the abundant information presented by Dr. Kirschbaum and Dr Syverton as to the multiplicity of factors which enter into the ultimate determination of whether or not, or when, leukemia occurs provides one with the basis for setting up a number of hypotheses with respect to human experience that are very stimulating to an epidemiologist. Obviously, the long, generation time of man enters into this picture and makes the direct execution of such experiments rather difficult, but I still think that an appropriate, well-devised study might yet be informative.

I was very much interested in Dr Syverton's views concerning antibody response to some of these viral agents in their native host, implying that this was possibly because the viral agent itself contained the host protein. I am wondering whether it might not also be due in part to the fact that these infections may be quite commonly congenital I am thinking now of the virus of lymphocytic choriomeningitis in the mouse. A mouse infected after birth develops antibodies to the virus in the usual fashion, whereas the mouse infected in utero, that is, congenitally, harbors the virus throughout its life but never develops antibody In this case, apparently, the virus, having been present in the developmental stage, does not elicit the customary response to foreign protein. Would this apply to leukemia?

DR SYVERTON: It seems to me that four possibilities deserve consideration:

1. An agent and its specific antibody can coexist with any free agent or virus being neutralized by free antibody. This state of coexistence of antibody and infectious agent, known as premunition, is exemplified by psittacosis in its natural host, a psittacine bird. The bird commonly shows apparent recovery from clinical disease, but continues to carry psittacosis virus in the presence of specific antibody. This silent or inapparent infection continues for variable periods of time until some adverse circumstance such as inclement weather, intercurrent infection such as salmonellosis, starvation, or other reason upset the resistance of the bird, resulting in recurrence of clinical disease.

2. A second category is exemplified by acquired tolerance in which exposure to an agent or antigen before or immediately after birth results in loss of the host's ability to react to that same agent upon reexposure later in life.

3. A third type is the liberation by certain agents of soluble antigens which persist widely distributed in the body and result in immune paralysis (fixed antigen prevents effective antibody formation by the host, the antibody-forming capacity of the host is blocked).

4. A fourth category is the case of agent, virus, or otherwise, so intimately associated with host tissues as to become essentially inseparable and to persist in what appears to be host nucleoprotein.

DR. KIRSCHBAUM: Although antibodies have not been demonstrated, Dr Syverton, is it not true that as mice age they become resistant to the development of mammary cancer following introduction of the agent. Has anything been done to determine whether neutralizing antibodies are present in the serum of these older animals?

DR. SYVERTON. To my knowledge, no.

DR KIRSCHBAUM Would you suspect there may be neutralizing antibodies?

DR SYVERTON A reasonable, working hypothesis is the concept that animals acquire before or at birth a variety of agents that maintain and remain dormant unless activated by provocative measure

DR KIRSCHBAUM. Dr Fox, were you suggesting that, after the agent became available to the fetus, the fetus was desensitized, and then in later life the individual was able to form antibodies to the agent?

DR FOX Yes, essentially what I was suggesting is that, because of contact with the agent during the fetal period, the agent in effect was not a foreign protein to that particular individual, although it might be to other members of the species

DR KIRSCHBAUM That seems not to be applicable to the mammary tumor agent because it is not available to the embryo That is, the agent cannot

be obtained from the embryo. The mammary tumor agent is obtained postnatally.

DR. SYVERTON: Is it not true that the same applies to other species within the first few days of life?

DR. WOOLLEY: That was the point I would like to stress, too. Apparently for the first few hours after birth, the animal is similar to the embryo in some respects. Dr. Syverton, would you discuss the time of fixation of viruses to tissue in relation to the appearance of disease at a later date.

DR. SYVERTON: It is not clear to me what you have in mind. If you refer to the incubation period of Shope rabbit papilloma, papilloma virus demands direct contact with the stratum germinativum of the epidermis to evoke infection For example, when papilloma virus is inoculated by a scratch that is made to extend over the juncture of skin and mucosa and onto the mucosal surface, papillomatous proliferation is limited to where contact was made with basal cells of epidermis, the papillomatous growth stops abruptly at the mucosal juncture. As for the incubation period of Shope rabbit papilloma, virus once brought into contact with basal cells requires from seven to forty-two days before yielding evidence of its presence by the development of small epithelial excrescences which become papillomas or warts. Once that incubation period has been achieved, cells reduplicate quite rapidly and continue to do so for from six weeks to about three months, at which time multiplication ceases and the virus tumor remains in its stationary phase. Up to and during the stationary phase in the tumor's developmental cycle, virus in varying amounts can be demonstrated in the papilloma cells. It is not unlikely that virus recovered during the stationary phase represents preformed virus kept intracellularly for weeks or months Papilloma virus is a most resistant virus, as measured by heat inactivation, desiccation, or other adverse circumstance The final stage in the developmental cycle, the stage of regression, is manifested in one of two ways as the involutionary phase when the lesion becomes dried, atrophic, and sloughs. The skin site heals, leaving little or no evidence of pre-existing tumor Contrariwise, the regressive state may be manifested by renewal of proliferative capacity to result in an epidermoid carcinoma which infiltrates underlying tissues, metastasizes, and kills the host Is it this well-established sequence that you had in mind, or was it the relationship of papilloma virus to Rous's V-2 carcinoma? Virus was demonstrated over several years in this transplantable tumor while maintained in Dr. Rous's laboratory Evidence for the presence of virus by antibody production was lost after many generations It is not clear whether the virus under consideration represented a passenger virus or a virus of significance in the pathogenesis of the V-2 carcinoma

DR WOOLLEY. What happens to the virus in the end organ, during this long period?

DR. SYVERTON: I do not know. Mammary tumor virus obviously must be present, since it is recoverable both from mammary tissues and a variety of other tissues during that time.

DR. BURCHENAL: Are there any insect or fowl vectors for the virus of the Shope papilloma in the wild rabbit?

DR. SYVERTON: The natural mode or mechanism for infection of the cottontail rabbit by papilloma virus is not known It is known that lesions, under natural conditions, occur chiefly on head, face, buttocks, and legs This anatomic distribution suggests that the cottontail acquires virus from its burrow. Dr. Shope has attempted, by ingenious approaches, to find the answer, even studying ground toads, ground owls, and other natural inhabitants of burrows It is my understanding that leukosis is a respiratory infection. Certainly, birds in the same enclosure contract the disease

DR BURCHENAL. But no insect vectors are known?

DR. KIRSCHBAUM: It occurs to me that virologists have the happy faculty of believing that virus is present even though one cannot demonstrate it It has been demonstrated that if the mammary tumor agent was not made available to mice of the high-tumor C3H strain by foster-nursing, as many as 30 per cent of the animals developed mammary cancer, and from these mammary cancers, the mammary tumor agent could not be demonstrated, using the methods that are ordinarily used to demonstrate it.

*In this case, the agent is said to be present because there is a method to* demonstrate it. If the mammary tumor agent is excluded by foster-nursing and then mammary cancer is induced by the administration of methylcholanthrene and it does not appear when one extracts the mammary tumors and injects the most appropriate strain of test animals, one must come to the conclusion that in carcinogen-induced mammary tumors of mice the agent is not acquired if it is not present at the outset There may be other methods of demonstrating the agent, but at least tentatively I have to come to the conclusion that it is not demonstrable under these conditions

DR WOOLLEY Are you referring to the same morphologic tumor when you speak of the carcinogen-induced tumor?

DR KIRSCHBAUM: I would say that in about 95 per cent of methylcholanthrene-induced mammary tumors there are areas of carcinoma. There are also areas of squamous metaplasia One might feel that this squamous metaplasia indicates that this is a different kind of cancer and that there is no agent found because this is not the kind of mammary cancer that occurs spontaneously, but I do not think one can brush aside the findings of Heston with that argument He finds mammary cancer with histology typical for the spontaneous neoplasm, although exhaustive experiments reveal no agent It seems to me that the agent

accelerates the onset of the disease. There are other potent factors, and the agent is not infectious in the usual sense but is an accelerating factor.

DR. SYVERTON: *I follow your line of thought. Certainly, it is not known why some people harbor xanthelasma and xanthoma tumors; the presence of such tumors is of significance to the attending physician.*

DR. BURDETTE: One of the things you mentioned in your talk a moment ago was that masking of viruses may sometimes be explained on the basis of the small number of particles present. It occurs to me that this removes the masking of viruses from the occult into something which may be tested. Would you comment?

DR. SYVERTON: *The term "masking" has been used in description of* biological findings to indicate: (a) a latent, noninfectious form of virus which may or may not give rise to discernible antibodies, (b) inhibition or "masking" of an infectious agent or virus by specific antibody, and (c) a quantitative relationship of viral particles to susceptible cells which demands a minimal number of particles for host transfer, as has been emphasized adequately by Beard. Bryan, at the National Cancer Institute, has provided convincing examples of why the term "masking" should not be used. He found that terminal dilutions of chicken tissue containing Rous sarcoma virus can result in the production of a tumor which eventually kills the host fowl. Yet, from such tumors, virus may not be recoverable. The more concentrated of successive twofold dilutions of the same chicken tissue induces tumors which contain approximately the same amount of virus as the original source tumor. Failure to demonstrate virus in tumors provoked by terminal dilutions is subject to interpretation as absence of virus or masked virus when antibodies in recipient hosts are demonstrated Bryan's studies have shown that a critical number of viral particles are essential for induction of a tumor and that this number varies from one virus tumor to the next

DR BURDETTE· Dr Syverton, would you hazard any comments about the manner in which tumor viruses act within the cell, whether actually this is only a matter of macromolecular structure, since an agent such as polyvinyl pyrrolidone has been shown by Hueper to be tumorigenic, and cellophane, as well as other polymers, will produce tumors, as shown by Oppenheimer and others, or whether, as Darlington has suggested, the way these viruses act is by entering the cell and acting through competition with essential cellular systems

DR. SYVERTON. *I am glad to comment if you will permit me considerable* latitude. Let us assume that a mammalian cell consists of a bag of enzymes and genes subject to rearrangement, replacement, and variable functions Infection by a virus, which is nucleoprotein foreign to the cell, introduces a new battery or bag of enzymes to take over and convert the cell into a factory capable of pro-

ducing, within predictable periods of time, an abundance of new nucleoprotein or virus. The time required for this process for bacteria can be as short as twenty minutes, for poliomyelitis, about eight hours; for many viruses, much longer. The result is production and release of a vast number of newly formed particles of nucleoprotein, new virus In this process, virus, the product, acquires materials from the host cell and in turn, upon transfer to a new host cell, alters its enzymic productive capacity.

DR. BURDETTE: Do you think this is a sort of transduction? Is that what you are implying?

DR. SYVERTON: Yes, but transduction signifies transfer between cells of a new property which may be biochemical, physical, or genic. Transfer by transduction is limited to a single characteristic. More nearly what I had in mind is the lysogenic relationship of bacterium and its bacterial virus, since by that relationship the bacterial cell maintains and transmits virus by its genic mechanism to its bacterial progeny.

DR. KIRSCHBAUM: Dr. Syverton, would the results of experiments with the Shope papilloma not suggest that the virus is responsible for the initiation of the neoplasm, but that continuing growth is not virus-dependent?

DR. SYVERTON: The findings can be so interpreted. An adequate explanation is not known for the disappearance of papilloma virus from tumor during its regressive stage inclusive of the carcinomatous lesion. Of examples acceptable as virus tumors, certainly the epidermoid carcinoma that follows the virus-induced papilloma has weak supportive evidence for a virus causation.

DR. GROSS Dr. Kirschbaum remarked about nonviral etiology of certain types of mammary tumors You must keep in mind that our means at the present time to establish the viral etiology of a tumor consist mainly of bioassay; we inject cell-free extracts into susceptible animals and observe animals for the development of tumors We know that in many instances the extract does contain virus and yet no tumor results Never can we state that the extract does not contain the virus just because the injection did not result in the development of a tumor It is quite possible, in fact, perhaps probable, that there are several types of mammary tumor viruses

The fact that some mice may still develop mammary tumors following foster-nursing suggests that such tumors may be transmitted just like the leukemic agent, directly through the embryo

DR. BURDETTE Do you have any evidence that another virus is involved in the causation of mammary tumors, or is this only speculative?

DR GROSS I have no evidence whatever, but I assume that not all mammary tumors are caused by the same agent, and that not all of them are transmitted through the milk

accelerates the onset of the disease. There are other potent factors, and the agent is not infectious in the usual sense but is an accelerating factor.

DR. SYVERTON: I follow your line of thought. Certainly, it is not known why some people harbor xanthelasma and xanthoma tumors; the presence of such tumors is of significance to the attending physician.

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*Arthur Kirschbaum, PhD, MD*

# ETIOLOGY *of* LEUKEMIA

Leukemia was recognized as a distinct clinical entity more than one hundred years ago, but its relationship to lymphosarcoma and other neoplasms remained quite obscure. Most of the work was descriptive until approximately 1908, when Ellermann,[27] working on leukemia in the domestic fowl, found that this disease can be transmitted to susceptible animals by the inoculation not only of cells but also of cell-free extracts, at this time, the possibility of the viral etiology of leukemia became apparent. It has taken more than forty years before the work of Gross[35] has made it seem likely that mouse leukemia may also have a viral etiology.

The first experimental study in mammals appeared in 1927 when a group working in Holland, Snijders[39] and Tio Tjwan Gie,[15] described leukemia and lymphosarcoma in a homogeneous stock of guinea pigs. These diseases could be transmitted to closely related animals by inoculating intact viable cells, the introduced cells grew at the site of inoculation to form a tumor, even though these cells were derived from the blood stream. Thus, these workers demonstrated that leukemia cells of the guinea pig may grow as a tumorous mass. When the cells of local tumors were inoculated intravenously into closely related animals, the recipients developed leukemia.

In 1929, Richter and MacDowell[30] described leukemia in the C58 strain of mice. This is a highly inbred strain in which 90 per cent of the animals develop lymphatic leukemia, and these workers began a systematic study on the genetics of this disease. One of their most important contributions was the demonstration that nongenetic as well as genetic factors determine the genesis of leukemia. Although 90 per cent of the animals of this genetically homogene-

DR. BURDETTE: In closing, Dr. Syverton, would you care to set up some rules for proving that a given virus is responsible for a given tumor Would you alter Koch's postulates in any way?

DR. SYVERTON: It has proved difficult to formulate rules to prove a given virus responsible for a given tumor. Evidence accumulated by Beard and his associates for erythromyeloblastosis virus as the causative agent of erythromyeloblastosis of chickens is conclusive. Yet, if one examines the origin of tumors of mice, or the rabbit papilloma-to-carcinoma sequence initiated by Shope's rabbit papilloma virus, the problem is difficult. In each instance, the evidence or findings are subject to the interpretation that the causative agent consists of indistinguishable host and virus constituents. Finally, as Rivers pointed out some years ago, Koch's dicta or postulates can be applied to virus infection.

DR BURDETTE: Later you will have an opportunity to make additional comments on the proofs offered for the viral etiology of leukemia.

The relationship of the chemical and physical leukemogenic agents (carcinogenic hydrocarbons, estrogenic hormone, and ionizing radiation) to the virus remains to be determined. Do these nonviral agents merely act by way of a filterable agent? In other words, is a filterable agent the mediator for the carcinogenic hydrocarbons and x-irradiation?

Thymoma (lymphosarcoma) of the mouse has attracted considerable attention because extirpation of the thymus has reduced the incidence of leukemia in certain high-leukemia stocks. In strains of mice susceptible to the induction of leukemia by carcinogenic hydrocarbons or x-rays, thymectomy may also reduce the incidence of the induced disease. There is something definitely peculiar about the thymus as far as the genesis of leukemia is concerned. Perhaps the environment provided by the thymus may be responsible for the leukemic transformation of cells which invade the thymus. Injection of thymoma cells intravenously into closely related animals may result in the appearance of systemic leukemia.

Systemic leukemia frequently is characterized by large lymph nodes, spleen, thymus, and liver. There is considerable variation in mouse leukemia from case to case, both histologically and etiologically Some investigators compare mouse leukemia and human leukemia, since the same forms of lymphomatous disease are seen in the mouse which are seen in the human species. I am inclined to think that the disease is so similar in the two species that perhaps we should not speak of *mouse* and *human* leukemia, but of the types, such as granulocytic and lymphocytic

We have emphasized the thymus as a focus of origin of leukemia However, the mesenteric lymph nodes may become lymphomatous, and metastasis to the liver from such a lymphosarcoma may occur. It is to be emphasized that leukemia in the mouse is quite variable. Considerable variation may be encountered in hybrids, but the disease tends to be more uniform in inbred strains of mice. On occasion, some question arises as to whether a lesion is hyperplastic or neoplastic. Some lesions of this type are not transplantable, and transplantation of mouse cells with progressive growth is a biologic test of neoplastic activity. Kaplan recently showed that early in the genesis of thymomas they may not be transplantable, whereas subsequently they transplant readily into genetically similar hosts Chlorotic leukemia may prove very valuable in testing such agents as busulfan In chronic, granulocytic leukemia, mice may have leukocyte counts as high as one million per cubic millimeter and become intensely anemic, and this disease may become acute terminally as in the human subject.

In strain A, although highly susceptible to the induction of pulmonary tumors, no leukemia was induced when methylcholanthrene was fed Therefore, the pulmonary tissue is susceptible to the action of the carcinogen, whereas the lymphoid and myeloid tissues are resistant In the DBA strain, on the other

ous stock developed leukemia, approximately 10 per cent remained leukemia-free. The progeny of this 10 per cent, however, did develop leukemia, the incidence being 90 per cent, suggesting that the 10 per cent nonleukemic were genetically similar to the 90 per cent which developed the disease. Therefore, it was assumed that nongenetic factors were responsible for the nonappearance of the disease in 10 per cent.

The next most important step was the observation by Furth and Furth[31] that leukemia may be induced in low-leukemia strains of mice by exposure to x-rays. This was the first experimental demonstration that the disease may be induced in animals which otherwise remain leukemia-free. The unfortunate events in Hiroshima demonstrate that ionizing radiations may induce the same disease in the human species as well,[32] so that leukemia of mouse and man may have a common etiology.

Mider and Morton,[33] in 1939, showed that the carcinogenic chemicals which induce various neoplasms quite readily in mice may, in certain genetically susceptible strains, cause the appearance of leukemia. This is further evidence that leukemia in mice is a neoplasm; chemicals which induce obvious cancers also induce leukemia Transplantation studies also indicate that leukemia of mice is a neoplasm because leukemia in mice transplants as do the other neoplasms of this species

A third leukemogenic agent, estrogenic hormone, was discovered by Lacassagne,[14] in France, and this work was expanded by Zondek, and by Gardner and Dougherty[38] in this country. This finding is rather difficult to reconcile with the possible etiology of the disease in man. However, related steroids from the adrenal cortex may modify the progress of leukemia in the human species, so that there may then be some fundamental link between the induction of leukemia in mice by estrogenic hormone and the appearance of the disease in man

Recently, the development which has attracted the most attention is the discovery of a possible biologic leukemogen, a filterable agent which can be obtained not only from leukemic tissue of mice, but also from the embryonic tissue of high-leukemia strains When this material was inoculated into certain low-leukemia strains, then the incidence in recipients was higher than in controls. Drs Gross and Woolley will discuss this work at length, and also Dr Syverton has reported on the viral aspects of etiology

We have mentioned that leukemia may appear spontaneously or be induced. I would like to emphasize that we cannot generalize about these agents which induced leukemia, they are operative depending on the genetic soil Not all mice are susceptible to the induction of leukemia by carcinogenic hydrocarbons, not all mice are susceptible to the induction of leukemia by estrogenic hormone, and not all mice develop leukemia spontaneously. Genes determine the response of the host. Both genetic and nongenetic factors are involved

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hand, another highly inbred strain, feeding the same carcinogen results in the induction of leukemia, whereas pulmonary tumors are not induced. This suggests that the target tissue determines the response to the leukemogen. Gene-determined, systemic metabolism of the carcinogen does not determine leukemogenesis, but something within the tissue itself does. The site of gene action is thus the target tissue. Examination of multiple tumors in the mammary glands of strain A mice, carcinogen-induced, reveals hundreds of foci where the carcinogen acts, but the lymphoid tissue does not respond at all.

Experiments to illustrate that the site of gene action is in the target tissue are of interest. The Bagg, albino strain is susceptible to the development of pulmonary tumors if urethan is given, urethan being the carcinogen. The DBA strain is urethan-resistant. Only an occasional tumor nodule appears. If the two strains are crossed, a susceptible $F_1$ hybrid results, this host being susceptible to the induction of pulmonary tumors. Prior to the administration of urethan, the pulmonary carcinogen, normal lung tissue from newborn mice of the susceptible parent stock, was grafted into the subcutaneous tissue of one ear, and newborn lung tissue from the resistant parent strain into the other ear. Thus, in an $F_1$, hybrid host susceptible to the induction of pulmonary tumors were normal lung tissue grafts from susceptible and resistant strains. After the grafts had taken, the animals were given the carcinogen. Tumors appeared in situ in the host, and in the susceptible tissue grafts (12 of 17). One small nodule was seen in a single, resistant, tissue graft (1 to 17). This illustrates that the site of gene action is in the target tissue and that the host, in this case susceptible, did not alter the resistance of the resistant tissue.

The same principle holds for leukemia. Law has crossed mice with a high incidence of leukemia to those in a strain having a low incidence of leukemia to obtain $F_1$ hybrids which were thymectomized. Removal of the thymus inhibited the development of leukemia, although these $F_1$ hybrids were otherwise susceptible. If thymus from the high-leukemia parent stock was grafted into $F_1$ hybrids early in life, then the $F_1$ hybrids developed leukemia. On the other hand, if the thymectomized, $F_1$ hybrids received grafts from the low-leukemia strain, then leukemia did not develop.[69] He then transplanted the leukemic cells which appeared. If the leukemia had arisen from the thymic graft of the parental stock, then this leukemia should have been transplantable not only into the parental stock from which the tissue came, but also into $F_1$ hybrids. The latter was the case. In other words, on the basis of transplantation experiments, Law had excellent evidence that the leukemic cells originated not from the thymic graft, derived from the high-leukemia strain, but from the $F_1$, hybrid host. Histologic examination revealed that the grafted thymus was reconstituted by infiltration of cells from the host. Although it did not become leukemic, the grafted thymus induced leukemic transformation of host lym-

phocytes which had invaded the graft. In other words, the grafted thymus of the high-leukemia strain represented a leukemia-promoting environment.

Kaplan and associates[44] found that thymectomized mice do not develop leukemia following irradiation because the thymus has been extirpated. If the thymus is grafted into these animals after irradiation, then leukemia develops, indicating that the thymus responds to some secondary factor following x-irradiation. This demonstrates that exposure of the thymus itself to x-rays is not necessary for the induction of leukemia.

Approximately 80 per cent of mice of the C58 strain develop leukemia spontaneously. If these animals are fed methylcholanthrene, leukemia occurs no earlier, and the incidence is ultimately no higher than spontaneously. In other words, although the animals are susceptible to spontaneous leukemia, they do not respond to the leukemogenic action of the carcinogen. Susceptibility to the spontaneous development of leukemia does not imply susceptibility to the leukemogenic action of the carcinogen. Although a low-leukemia strain, some leukemia does appear in DBA mice, more in the female than in the male animals. When this strain receives methylcholanthrene either by skin-painting or feeding, leukemia develops. Eighteen skin-paintings were given over a period of six weeks to a group of DBA mice. When the dose of carcinogen was low, then thymectomy inhibited the induction of leukemia. If, however, the dose of methylcholanthrene was doubled, then thymectomy did not inhibit leukemogenesis. It would appear that the thymus is preferentially susceptible to the leukemogenic action of methylcholanthrene, but other lymphoid tissue is quite susceptible when large doses are given.

If a strain of mice is susceptible to the independent leukemogenic action of certain agents, and if these agents are given in combination, leukemia appears earlier and in higher incidence. When whole-body x-irradiation, 200 r, was supplemented with 5 μg estradiol dipropionate (estrogen), weekly after x-irradiation, leukemia appeared earlier in Balb c mice, and the incidence was higher than if the agents were administered independently. The thymus was the primary site of response. To demonstrate the specificity of this synergism, mice of this strain were given methylcholanthrene. Out of 535 mice, only 6 developed thymomas, whereas 18 of 48 mice which received the x-rays plus estrogen developed thymomas.

Age has a striking influence on susceptibility to leukemogenic action. Most strains are susceptible to leukemogenic agents only if they are treated early in life. In the high-leukemia C58 strain, we see that leukemia appears earlier in female than in male mice, but the ultimate incidence is not much different. Three different crosses were made between the high-leukemia C58 strain and three low-leukemia strains, the CBA, C57, and NH, all of which are low-leukemia strains. The leukemias appeared in female mice earlier than in male mice in all crosses. The age of occurrence of leukemia in hybrids is much later

than in the pure C58 stock. The $F_1$ hybrids have a longer life expectancy and develop leukemia late in life. In reciprocal crosses between the C58 and NH strains, leukemia appears later in life if NH female rather than male mice are used.

In our laboratory, the work of Woolley on effects of cortisone on leukemogenesis has been confirmed. High-leukemia C58 mice were given large doses of cortisone at monthly intervals for several months beginning at 2 months of age. Although the ultimate incidence of spontaneous leukemia was the same, leukemia appeared significantly later in life Cortisone inhibited induction of leukemia by methylcholanthrene

MacDowell found that if C58 female mice were foster-nursed by animals of the StoLi strain, their life expectancy was greater Not only did these animals live longer, but also the incidence of leukemia was lower. A factor in the breast milk reduced the incidence of leukemia

We think of carcinogens as initiating leukemogenesis. We do not think usually of carcinogens as having any effect upon the host. If the Gardner lymphosarcoma of C3H origin is transplanted within this strain, growth is progressive in 100 per cent of the recipients. If, however, this lymphosarcoma of C3H origin is grafted into a foreign strain, DBA, there is temporary growth with regression, and then the animals resist transplantation. We have done this as many as eleven successive times. After each inoculation of lymphosarcoma tissue, there is absolutely no growth The animals appear to be permanently immune. If immune mice were skin-painted with methylcholanthrene, and then challenged by tumor inoculation, growth was progressive in one-third of the animals After growth in methylcholanthrene-treated DBA mice, the progressively growing tumor transplants as it did originally, indicating that there is no change in the tumor itself, but more likely a change in the host *as a result of treatment with methylcholanthrene.*

In summary, we can say that genetic factors influence the development of spontaneous and induced mouse leukemia. Leukemogenic agents include ionizing radiation, chemical carcinogens, and estrogenic hormone Certain influences, for example, administered cortisone, may modify the growth of leukemic cells, although initiation of the disease is not inhibited Other factors actually may inhibit leukemogenesis (caloric restriction, administered androgen, thymectomy)

## DISCUSSION

DR BURDETTE: Dr. Sprague, would you comment on similarities between leukemias in mice and leukemias in man?

DR. SPRAGUE: I thought there was striking similarity between the morphology of leukemia in these animals and what we see in man This seemed to

be particularly true of the acute lymphocytic and chronic granulocytic leukemia that Dr. Kirschbaum showed.

DR. BURCHENAL: That was strongly evident. Was the leukemia shown the type tending to respond to arsenic?

DR. KIRSCHBAUM: Yes, if we transplant chronic granulocytic leukemia and administer arsenite, in some instances we may actually prevent the growth of the transplanted cells

DR. BURCHENAL: In other words, one might say morphologically and biochemically it resembles the granulocytic strain in man. Arsenic would affect the chronic disease in man as well

DR KIRSCHBAUM: That is right.

DR. TILL: Did I understand you to say that transplanting this chronic granuloleukemic disease turned it into acute leukemia?

DR KIRSCHBAUM: That is the impression we had, judging from the morphology. In certain instances, there was a shift to the left just prior to death but not invariably. It is true, too, that if we take one of these chronic leukemias and transplant it many times so that it becomes more acute, then there is a progressive shift to the left with less and less differentiation on passage through multiple hosts

DR SCHLOSSER Do androgens have a similar effect?

DR KIRSCHBAUM· In the case of methylcholanthrene-induced leukemia in DBA mice, the administration of androgen results in a decreased incidence of induced leukemia, and castration has been demonstrated in AKR mice to increase the incidence of spontaneous leukemia We also have evidence that the incidence is higher in females than in males I would like to mention the work of Kaplan and of Gardner on radiation-induced leukemia The administration of androgen prevents induction At least, there is a markedly lower incidence in irradiated animals that also received androgen, indicating that androgen is antileukemic. One thing that does disturb me is that I am not sure we have been as careful as we should have been in controlling the weight of these animals In all this therapy one wonders about debilitating effects, whether caloric restriction and inanition resulting from the administration of these agents is at least contributing toward the effect that is obtained In cortisone-treated animals we have observed some decline in weight after the administration of these rather large doses, although the ultimate weight may not be altered very much Perhaps Dr Woolley would like to comment on this.

DR WOOLLEY Using the strain AKR, we found that there was a depression in weight after administration of cortisone which lasted almost two weeks, followed by a gradual gain in weight until the time of the next monthly treatment, when the animals were up to full weight If one prepared a curve of weights immediately before therapy, there was no depression If one prepared a curve of weights soon after treatment, then there was a depression in body weight.

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agents. For example, the right combination of cortisone and x-irradiation will alter natural resistance of any species.

Dr. Kirschbaum, do you consider the agents, steroid hormones and x-irradiation, provocative agents or causative agents of leukemia? If the various types of leukemia are distinctly different diseases, one could suspect separable causative agents.

DR. KIRSCHBAUM: In a single inbred strain of mice, granulocytic and lymphocytic leukemia may appear. One may be seeing different manifestations of the same disease with the same provocative agent, or this may represent a strain susceptible to the induction of leukemia by different agents, perhaps different viruses. Granulocytic leukemia, chronic in FB mice, never appears before 400 days of age; by treatment with carcinogenic hydrocarbons, the disease will appear earlier. It so happens that this strain is susceptible to the spontaneous disease and also susceptible to carcinogenic induction. I do not know what effect estrogenic hormone or x-rays would have. Is that what you have in mind?

DR. SYVERTON: The experiences you cite are examples of extrinsic agents influencing innate resistance. Precisely what takes place when methylcholanthrene, x-irradiation, or other extrinsic factor is operative is not known, but each acts as a provocative carcinogenic agent.

DR. KIRSCHBAUM: At this time I would like to make a distinction between transplanted and spontaneous leukemia. Dr. Syverton has mentioned resistance; he implied that this resistance is something directed both against the transplanted and spontaneous disease, and that x-rays and cortisone will facilitate or permit the growth of human neoplasms in animals Also, I know that Dr. Burchenal is transplanting leukemia of other species into hamsters. To me, the important biological characteristic of leukemia is its progressive growth. However, the spontaneous disease differs from the transplanted disease in that one can immunize against transplanted, but not against spontaneous disease. I suspect that spontaneous mouse leukemia is more like human leukemia than human leukemic cells growing in a hamster

DR. BURCHENAL: In hamsters we have never succeeded in growing leukemic cells that we were sure were human, leukemic cells I agree that spontaneous leukemia in the mouse or in any other animal is much more like spontaneous leukemia in man than transplanted leukemia, but, as Dr Kirschbaum knows, it is easier to work with transplanted leukemia for certain technical reasons Such studies are usually done with the hope that an analogy between the behavior of transplanted leukemia in the mouse or in any other mammal and human spontaneous disease may be found. A great deal of my interest is in acute leukemia and transplanted leukemias As Dr. Kirschbaum has mentioned, no matter how chronic it may be in the mouse, the spontaneous disease usually tends to become acute after a few transplant generations, and there has been some similarity between transplanted leukemias.

DR. KIRSCHBAUM: Have you depressed the body weight by restricting caloric intake? I think this should be done, and we have not done it.

DR. WOOLLEY: We have not done that either. You will recall that Dr. Furth performed a caloric-restriction experiment, and caloric restriction prolonged the time of appearance of leukemia Also, the ultimate incidence of leukemia was probably reduced by caloric restriction alone.

DR KIRSCHBAUM: Of course, there is some difference in reducing the body weight of animals by one-third temporarily and keeping them at that level. Here we have a situation where there are fluctuations in body weight, depression after cortisone, and return to what is considered to be a normal weight for the strain.

DR. WOOLLEY: It seems to me the hormonal factor can be separated quite easily. Testosterone, a protein anabolic substance, has been used by us (unpublished data) to reduce the incidence of leukemia in the high-leukemia strain, AKR.

DR. KIRSCHBAUM: Another argument against the caloric factor is that animals treated with estrogenic hormone may look debilitated, but still develop leukemia.

DR. WOOLLEY. I agree. Results must be compared experiment by experiment and strain by strain. In our experience, the incidence of leukemia in the susceptible, AKR strain has been reduced, although an increased incidence of leukemia in resistant animals has been reported. ACTH is a substance which presumably acts by means of cortisone production, causing lymphoid tissue destruction.

DR SPRAGUE· In acute leukemia, I would like to ask what percentage of these animals show that change, and if a similar change is seen following treatment with either arsenic or urethan

DR KIRSCHBAUM· I cannot give you any figures, but the important thing is that there is a relationship between chronic and acute leukemia. Invariably, these chronic granulocytic leukemias become acute if they are transplanted many times

DR. BURDETTE Dr. Syverton, will you comment on the action of leukemogens in relation to host resistance?

DR. SYVERTON. Among factors which influence disease irrespective of one type are genetic make-up, age of the host, and hormonal balance These intrinsic factors determine natural, innate resistance as opposed to natural, innate susceptibility. For example, poliomyelitis and measles viruses are highly infectious for man, whereas an enormous amount of these viruses can be administered to other species, such as dog or rat, without effect Yet the clinical manifestations of both diseases in man are influenced by age, genetics, and hormones Why is an occasional person naturally resistant? Natural resistance for man and other species is subject to experimental manipulation by using a variety of provocative

*Ludwik Gross, M.D.*

# INFECTIOUS CONCEPT *of* LEUKEMIA

*(Based on Recent Experiments on Cell-Free Transmission of Leukemia in Mice)**

It has been suspected for many years that leukemia developing "spontaneously" in various species, including human beings, may be caused by transmissible viruses. Thus far, however, this assumption can be confirmed experimentally in two species only, namely, in chickens and, recently, also in mice. Ellermann and Bang (1908)[27] were the first to determine that chicken leukemia could be transmitted by inoculation of filtered extracts. The viral nature of mouse leukemia was revealed in our laboratory several years ago.[1, 39-41] Filtered extracts prepared from leukemic mice of high-leukemic strains, such as AK or C58, were inoculated into *newborn* mice of strains C3H, C3H (f), or C57 brown,[42] which are, for all practical purposes, free from leukemia. More than 30 per cent of inoculated mice developed typical generalized leukemia after a latent period varying from a few months to one and one-half years (Fig. 1). These results now have been confirmed by Woolley and Small[43] at the Sloan-Kettering Institute. In our experiments, the leukemic agent proved to be highly specific, requiring particular strains of mice for inoculation. Thus, the Bittner substrain of the C3H line proved to be susceptible, whereas the closely related National Cancer Institute substrain of the same inbred line proved to be substantially more resistant.[44]

*Aided in part by a grant from the Damon Runyon Memorial Fund

With regard to Dr. Syverton's question, Dr Furth tried to obtain myeloid leukemias in the AK strain by performing thymectomy, followed by irradiation. His idea was that since most of the leukemias were lymphatic, usually thymomas in that strain, the incidence of these leukemias was reduced markedly, allowing the animals to live to an age when they might develop the granulocytic type of leukemia if one removed the thymus.

DR. KIRSCHBAUM: I am not sure about the nature of the induction. I do know thymectomy has prevented the development of lymphocytic leukemia and the mice lived to develop granulocytic leukemia, which suggests the genesis of *the granulocytic and lymphocytic disease is different, also the primary locus is* in the thymus in only one of the two

DR SYVERTON: In their discussion, Drs Burchenal and Kirschbaum illustrated the influence of extrinsic and intrinsic factors upon the host As they point out, a given host under influence by one or several modifying factors can show widely variable manifestations of its disease

DR BURCHENAL: The other point to be brought out is that first or second generation transplants from spontaneous leukemia probably are much closer to the spontaneous form than those transplanted for several generations. Would you agree?

DR. KIRSCHBAUM: I would indeed MacDowell, in classical experiments, demonstrated that one can immunize by injection of normal cells of certain genetic type This immunization is against transplanted leukemia, but not against spontaneous leukemia nor from the cells of a spontaneous case In other words, one cannot immunize against the first transplantation of leukemic cells so that the first transfer generation would apparently be more like spontaneous leukemia than is the usual transplanted disease

DR BURDETTE Dr Gross, you speak frequently about the relation of viruses to the etiology of leukemia Would you comment on the maternal resistance factor in milk which Dr Kirschbaum mentioned? Do you regard it as a virus?

DR LUDWIK GROSS I do not have enough information on this factor. I would like to comment on the difference between spontaneous and transplanted leukemia First, I would like to question the terminology "spontaneous" because, if the work which we are doing is correct, there is no such thing as spontaneous leukemia in mice Spontaneous smallpox or spontaneous measles could then be compared to spontaneous leukemia What we call spontaneous would be leukemia naturally induced

DR KIRSCHBAUM This is purely a question *of terminology If there is a* virus which is responsible for the development of leukemia in mice without man's intervention, then virus leukemia is spontaneous leukemia

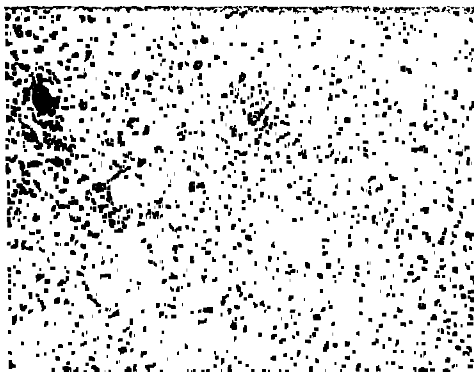


Fig 1 —Leukemic infiltration of liver This C57 brown/cd female, No 173, Exp

TABLE I

INOCULATION OF FILTERED (SELAS OR BERKEFELD) AK LEUKEMIC EXTRACTS INTO NEWBORN C3H OR C3H (f) MICE OF TWO SUBSTRAINS

| | FRESH EXTRACTS | | | HEATED EXTRACTS (65° TO 68° C ½ HR) | | |
|---|---|---|---|---|---|---|
| SUBSTRAIN | NUMBER INOCULATED | NUMBER DEVELOPED LEUKEMIA* | NUMBER DEVELOPED PAROTID TUMORS* | NUMBER INOCULATED | NUMBER DEVELOPED LEUKEMIA | NUMBER DEVELOPED PAROTID TUMORS |
| Bittner C3H | 138 | 32 (23%) | 24 | 73 | 0 | 1 |
| Bittner C3H (f) | 182 | 58 (32%) | 9 | 93 | 2 | 4 |
| Bittner total | 320 | 90 (28%) | 33 | 166 | 2 | 5 |
| NCI C3H (f) | 162 | 7 (4%) | 9 | 45 | 0 | 0 |

*Average age, months leukemia, 11, parotid tumor, 5

Recent experiments carried out in our laboratory, utilizing electron microscopy, ultracentrifugation, and filtration through gradocol membranes, suggest that the mouse leukemic agent is a particle having a diameter less than 100 mμ [45]

TABLE II

BASIC DIFFERENCES BETWEEN LEUKEMIA TRANSPLANTED (BY CELL GRAFT) AND INDUCED (BY INOCULATION OF CELL-FREE* EXTRACTS)

| | RESULTS OF INOCULATION INTO MICE OF | | | | | | |
|---|---|---|---|---|---|---|---|
| | THE SAME STRAIN (AK) | | SUSCEPTIBLE FOREIGN STRAIN (C3H OR C57 BROWN) | | | | |
| | NEW-BORN | ADULT | NEW-BORN | ADULT | INCUBATION TIME MONTHS | *LEUKEMOGENIC* TUMOR AT SITE OF INOCULATION | RESULTING LEUKEMIA CAN BE TRANSPLANTED TO |
| Transplantation of leukemic cells | Positive | Positive | Positive | Negative | ½ to 1 | Usually present | Donor strain |
| Inoculation of cell-free* AK leukemic agent | ?† | Negative | Positive | Negative | 3 to 24 | Absent | Recipient strain |

*Centrifuged 7,000 × g or filtered

†Possibly accelerated development of spontaneous leukemia Sufficient experimental information not yet available

TABLE III

TRANSPLANTATION OF LEUKEMIC CELL SUSPENSIONS (FROM SPONTANEOUS, TRANSPLANTED, OR INDUCED LEUKEMIA INTO ADULT MICE OF AK AND C3H STRAINS)

| LEUKEMIC DONOR | | RESULTS OF INOCULATION OF ADULT RECIPIENT MICE | |
|---|---|---|---|
| DONOR | ORIGIN OF DONOR | STRAIN AK | STRAIN C3H |
| AK | *Spontaneous* | *Positive* | *Negative* |
| C3H | Inoculation of AK leukemic cells* | Positive | Negative |
| C3H | Inoculation of cell-free AK agent† | Negative | Positive |

*AK leukemic cells grafted into newborn C3H mice cause leukemia in two to three weeks

†Following inoculation of cell-free AK extracts into newborn C3H mice, leukemia develops after three to twenty four months

Among the C3H or C3H (f) mice that have been inoculated in our laboratory with filtered leukemic extracts, some developed tumors of the parotid glands and/or subcutaneous fibromyxosarcomas instead of leukemia (Figs 2 and 3)

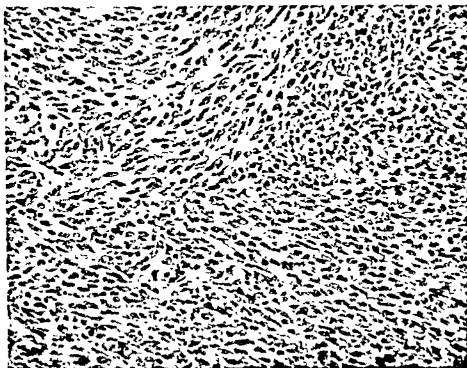


Fig 3 —This C3H (f) female, No 156 Exp 2644-L was inoculated when less than 5 hours old with a filtered (Selas 03) AK leukemic extract As a result, this mouse developed a subcutaneous spindle cell sarcoma in the right groin at 18 5 months of age (Hematoxylin and eosin magnification ×300 )

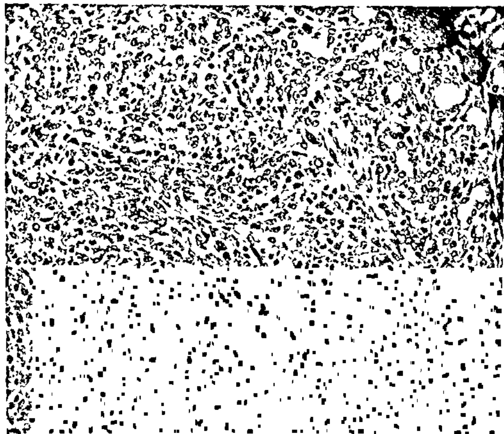


Fig 2 —Parotid gland tumor This C3H male, No 363, Exp 2709-C was inoculated when less than 15 hours old with a centrifuged (7,000 × g) AK leukemic extract, diluted 1 100 As a result, this mouse developed bilateral parotid gland tumors at 4.5 *months of age* (Hematoxylin and eosin magnification, ×315 )

extracts prepared from mouse leukemic tissues were inoculated into newborn mice of susceptible strains, conceivably leukemic cells contain several distinct oncogenic agents in certain forms of leukemia in mice. The fact that mixing the leukemic extract with ether[45] destroyed leukemogenic potency, leaving unchanged the extract's ability to induce parotid tumors or sarcomas, is consistent with the assumption that the leukemic extract contained several oncogenic agents and that the leukemogenic agent in such an extract was more labile and

TABLE VII

INOCULATION OF ETHER-TREATED LEUKEMIC EXTRACTS INTO NEWBORN C3H OR C3H (f) MICE

| NUMBER OF EXPERIMENTS | NUMBER OF MICE INOCULATED | NUMBER DEVELOPED LEUKEMIA | NUMBER DEVELOPED PAROTID TUMOR | NUMBER DEVELOPED SARCOMA |
|---|---|---|---|---|
| 6 | 53 | 1* | 6 | 2 |

*Chronic form of leukemia

therefore destroyed by the action of ether in vitro. Should the latter assumption prove to be correct, the presence of several oncogenic agents in the leukemic extract may not be due to a mere accident alone. Whatever the theoretical interpretation, the fact remains unchanged that leukemia in mice of either the AK or C58 high-leukemic lines is caused by a filterable and thermolabile agent which is transmitted under natural conditions of life from one generation to another directly through the embryo. Mouse leukemia, like chicken lymphomatosis, appears, therefore, to be an egg-borne virus disease.

## DISCUSSION

DR. KIRSCHBAUM: Dr Gross, you emphasize the fact that the virus must be introduced soon after birth if leukemogenesis is to proceed. How do you reconcile this with the fact that leukemia may be induced in mature animals of low-leukemia stocks by the administration of carcinogens, x-rays, or estrogenic hormones? In those situations, do you believe there is a virus available which mediates the action of the carcinogen?

DR. GROSS: It is very difficult to answer this question. I suspect that the leukemic agent is widespread, perfectly adapted in the host, causing no symptoms unless activated by such factors as x-rays or carcinogens.

DR. BURCHENAL: Dr Gross, you say that you think this virus is probably widespread. In what other strains of mice have you found it besides the AK and C58? Has it been demonstrated in other lines of leukemic or nonleukemic mice?

DR. GROSS: Thus far I have been able to recover the agent only from AK and C58 mice.

DR. BURCHENAL: Have you tried DBA mice? For instance, the DBA strain has a fairly low incidence of leukemia, but leukemia is readily induced with

When cell-free, centrifuged or filtered extracts then were prepared from the induced parotid tumors or subcutaneous sarcomas[46] and inoculated into newborn C3H mice, either parotid tumors, subcutaneous sarcomas, or leukemia resulted. This immediately raised the question whether filtered extracts prepared from leukemic AK or C58 donors contain a single agent capable of causing different types of tumors, or whether they contain a mixture of oncogenic agents.

TABLE IV

INOCULATION OF CELL-FREE EXTRACTS FROM INDUCED C3H LEUKEMIAS INTO NEWBORN C3H AND C3H (f) MICE

| NUMBER OF | NUMBER OF MICE | NUMBER DEVELOPED | NUMBER DEVELOPED | NUMBER DEVELOPED |
|---|---|---|---|---|
| | | | | |

‡Four developed spindle-cell sarcomas also

TABLE V

INOCULATION OF CELL-FREE EXTRACTS FROM INDUCED PAROTID TUMORS INTO NEWBORN C3H AND C3H (f) MICE

| NUMBER OF EXPERIMENTS | NUMBER OF MICE INOCULATED | NUMBER DEVELOPED LEUKEMIA* | NUMBER DEVELOPED PAROTID TUMOR* | NUMBER DEVELOPED SARCOMA* |
|---|---|---|---|---|
| 38 | 300 | 49 | 23† | 28 |

*Average age, months leukemia 14 parotid tumor, 6, sarcoma 15

†Of these, 6 developed parotid tumors and spindle-cell sarcomas, 2 developed parotid, submaxillary, and adrenal tumors

TABLE VI

RESULTS OF INOCULATION OF CELL-FREE SARCOMA EXTRACTS INTO NEWBORN C3H OR C3H (f) MICE

| NUMBER OF EXTRACTS PREPARED | NUMBER OF MICE INOCULATED | NUMBER OF MICE DEVELOPED LEUKEMIA | NUMBER OF MICE DEVELOPED PAROTID TUMORS | NUMBER OF MICE DEVELOPED SARCOMAS | AVERAGE AGE LEUKEMIA OR TUMORS DEVELOPED (MONTHS) |
|---|---|---|---|---|---|
| 36 | 316 | 8 | 6 | 9 | 7 |

It is conceivable that a single oncogenic agent can induce different types of tumors as well as leukemia On the other hand, it is also possible to assume that leukemia or certain other neoplasms contain several distinct oncogenic agents Only one agent may be pathogenic at a given time, even though the others also may be present in the same tumor This would explain why parotid tumors or sarcomas could be induced with leukemic extracts, and why, when Graffi and his co-workers[47] prepared filtered cell-free extracts from certain transplanted mouse tumors, such as the Ehrlich carcinoma, and inoculated them into newborn mice, leukemia resulted

Since tumors other than leukemia, such as parotid carcinomas or subcutaneous fibromyxosarcomas,[46] have been induced consistently when cell-free

extracts prepared from mouse leukemic tissues were inoculated into newborn mice of susceptible strains, conceivably leukemic cells contain several distinct oncogenic agents in certain forms of leukemia in mice. The fact that mixing the leukemic extract with ether[19] destroyed leukemogenic potency, leaving unchanged the extract's ability to induce parotid tumors or sarcomas, is consistent with the assumption that the leukemic extract contained several oncogenic agents and that the leukemogenic agent in such an extract was more labile and

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|---|---|---|---|---|
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*Chronic form of leukemia

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DR GROSS It is very difficult to answer this question I suspect that the leukemic agent is widespread, perfectly adapted in the host, causing no symptoms unless activated by such factors as x-rays or carcinogens

DR. BURCHENAL. Dr. Gross, you say that you think this virus is probably widespread In what other strains of mice have you found it besides the AK and C58? Has it been demonstrated in other lines of leukemic or nonleukemic mice?

DR GROSS Thus far I have been able to recover the agent only from AK and C58 mice.

DR BURCHENAL. Have you tried DBA mice? For instance, the DBA strain has a fairly low incidence of leukemia, but leukemia is readily induced with

methylcholanthrene. Therefore, if the virus must be present for the induction of leukemia by a physical or chemical agent, it should appear in the strain. On the other hand, if the virus is only an additional carcinogen such as irradiation or methylcholanthrene, any one without the other would work. Your hypothesis could thus be tested very well if you could demonstrate there is a virus present in DBA mice.

DR. GROSS: I have not performed experiments with DBA mice.

DR. BURCHENAL: I wonder whether leukemogenesis is dependent always on the presence of the virus, or can one agent cause it without the other: methylcholanthrene without the presence of virus, exposure to x-rays without the presence of virus, or the virus without the presence of, shall we say, cosmic rays or some other factor.

DR. GROSS: Much additional work is required before this can be answered adequately.

DR. SYVERTON: You referred several times to the use of temperature for inactivation. Have you attempted to use graded temperatures for differential separation to learn whether you are dealing with one agent or three distinct agents? It seems reasonable that multiple agents should be separable by a temperature differential for inactivation.

DR. GROSS: Yes. Such studies are in progress.

DR. SYVERTON: Do I understand that a temperature of 80° is required to inactivate all three viruses or agents?

DR. GROSS: Almost complete inactivation is obtained by heating to 65°-68° C for thirty minutes. However, parotid tumors occasionally may be induced with heated extracts.

DR. KIRSCHBAUM: Although you did not mention it today, Dr. Gross, you have taken adult C3H tissue, that is, tissue from a low-tumor strain and made extracts of that adult tissue, injected it into the newborn of the same strain, and obtained parotid tumors. How do you explain this on the basis of viral etiology? A second question is whether parotid tumors can be induced by the administration of cortisone alone.

DR. GROSS: It is true that, in a small number of animals, tumors may be induced by injecting C3H mice with extracts from normal C3H or normal C57 brown mice. I assume that the parotid tumor agent is present in many strains of mice. However, it is probably so adapted in the host that it causes no disease in the carriers. If cortisone is applied, one may occasionally induce parotid tumors in such hosts. Following a blind transfer into newborn mice, one can occasionally also induce such tumors.

DR. KIRSCHBAUM: I am still curious. If you can obtain parotid tumors by injecting adult C3H extracts, and if you feel these tumors appear only because you introduce the agent into the newborn, how can the agent be there? The animal does not develop the tumor unless you provide the agent. These are animals

*that have not been injected with the agent, and yet you are able to extract from* their tissue something which induces the neoplasm. It would seem to me that the element of nonspecificity enters here. Do you really have reason to believe this tissue has the agent?

DR. GROSS: Dr. Syverton, would you care to comment on this?

DR. SYVERTON: The problem as presented suggests that Dr. Gross is employing an approach which has been used not uncommonly to demonstrate the presence of a latent agent. That approach is to use a provocative injection or implantation of foreign material, such as tissue, starch, or chemical, to lower host resistance to a point where overt evidence of cell injury by the infectious agent is shown by clinical disease. Actually, I am arguing both for and against Dr. Gross's point of view here. For example, if the agent responsible for any one or more of the three tumors you describe were present as an indigenous, latent, virus infection, any inoculation may have served as provocative agent

DR GROSS: I assume that one can induce tumors in mice by blind passage Now, in the case of the parotid tumor agent in C3H mice, let us assume that many, perhaps all, C3H mice carry a small quantity of parotid tumor agent of low pathogenic potential Such low potential will not induce parotid tumors spontaneously. However, if one takes C3H normal tissue containing such an agent and injects it into a newborn mouse, its potential may be sufficiently increased to induce the disease in the injected host.

DR. BURDETTE· Are you saying, Dr Gross, that this is a quantitative and not a specific phenomenon—that the agent exists in all your animals, and when you inoculate them with extract, you are merely increasing their quantity?

DR GROSS You increase the pathogenic potential of the agent.

DR BURDETTE Would you define "pathogenic potential"?

DR. GROSS: By passing virus from one host to another, potency to induce disease is very often increased

DR. BURCHENAL. Does not such passage result in the enhancement of the virulence of the virus, presupposing recipient and donor are similar?

DR. SYVERTON There are several factors

DR KIRSCHBAUM C3H mice, according to your hypothesis, develop neither leukemia nor parotid tumors because they do not have the agent They develop these diseases if you introduce the agent from another strain at birth The thing I do not understand is how you can obtain this agent for inducing parotid tumors from adult C3H mice It would seem to me if such an agent for parotid tumors can be obtained similarly, you might obtain an agent for leukemia

DR WOOLLEY With the permission of Dr Gross I would like to discuss this matter We have used cortisone for chronic treatment of strain C3H mice and obtained parotid gland tumors Both C3H mice from the National Cancer Institute and C3H mice from Dr Gross's laboratory were treated with cortisone In the latter case, we secured a few parotid gland tumors, and we did not obtain

these tumors in the former. Apparently, the Bittner (Gross) subline is carrying a small amount of parotid tumor virus, and the other one, the NCI subline, is not. The amount of virus carried without concentration by inoculation into the newborn is probably not enough to lead to expression by tumorigenesis unless cortisone is used.

DR. BURDETTE: In any discussion of the interrelation between genetic susceptibility and the presence of viral particles within the cell, careful distinction must be made between the two. Attention so far has been focused on the existence and dosage of such particles within normal cells.

DR. WOOLLEY: Yes, some clarification is in order. Dr. Gross has just reported induction of leukemia in strains usually having a low incidence Perhaps the strain with low incidence will not easily support the leukemia virus generation after generation. Otherwise, it would be a strain with high incidence. An example of this would come from studies on the milk factor utilizing a strain with a low incidence of breast cancer, such as the C57 black stock which cannot support the milk factor virus generation after generation, at least in a high enough concentration to lead to mammary cancer

DR. KIRSCHBAUM. Dr. Woolley, you are implying that the virus is ubiquitous, present even in the C3H strain without being introduced from a foreign strain, so some nonspecific factor, such as cortisone or something which is responsible for the secretion of adrenal cortical hormone, will similarly induce the development of parotid tumors.

DR. WOOLLEY: In the first place, one must further define strain C3H. Sublines may differ in character considerably. Skeletal variation within the strain is one example

DR KIRSCHBAUM: Do C3H mice not develop parotid tumors unless injected with extracts at birth or unless they obtain cortisone?

DR WOOLLEY I do not know whether this is due to a resistance factor or not The fact is that in this particular experiment, where cortisone was given periodically throughout life, parotid gland tumors occurred in one subline and not in the other

DR KIRSCHBAUM If the virus is there, how important is its introduction at birth? Is this not a rather redundant procedure?

DR. GROSS It is very important

DR BURDETTE If my interpretation of the opinions of the participants is correct, most of the panel seems willing to accept the evidence that a virus is one of the factors which can be important in the etiology of leukemia in mice, but several members are still not quite convinced that this is always an essential factor requisite for the development of leukemia

*George W. Woolley, Ph D*

# FILTERABLE AGENTS PREPARED *from* LEUKEMIC TISSUE CAUSING LEUKEMIA *and* OTHER CANCERS *in* MICE*

During the past year, we have been investigating the relation of cell fractions prepared from leukemic tissues—centrifugates and filtrates—to the occurrence of cancer in mice These and other studies have been undertaken to help resolve the leukemia problem into its component parts and thus make its control more precise.

The methods of fractionation and injection have followed closely those developed by Gross.[49] Extracts of 20 per cent concentration prepared with physiologic saline solution from leukemic organs were centrifuged at 3,000 r.p m. for fifteen minutes, then at 9,500 r p m for ten minutes at 0° C. The supernatant fluid was used for inoculation or passed through a Selas 02 or 03 filter prior to use. All extracts were kept at 0° C and injected (0 05 ml ) subcutaneously within forty-eight hours. Newborn mice were used for inoculations, and most animals were less than 12 hours old at the time of inoculation. Following weaning, the mice were aged without breeding and observed for tumor occurrence [41, 50]

The occurrence of tumors appeared to be modified by the type of preparation used and by the genetics of the host receiving the preparation Of 31 prep-

*This investigation was supported in part by a research grant (CY-3784) from the National Cancer Institute of the National Institutes of Health, United States Public Health Service, and in part by a research grant from the American Cancer Society (T-47)

arations (20 of AKn/Gs origin and 11 of $C3H_f$/Gs origin), a total of 13 (6 AKn and 7 C3H) were active in inducing leukemias, tumors of the parotid gland, and sarcomas. The most active preparations were extracted from filtrate-induced tumors rather than from spontaneous tumors (Fig. 4). One such preparation, a filtrate, induced 4 leukemias out of 5 inoculated $C3H_f$/Bi mice (80 per cent) and 10 out of 17 $C3H_f$/Gs mice (59 per cent). More tumors appeared in $C3H_f$/Gs and $C3H_f$/Bi hosts than in $C3H_f$/Hu, C3H/Gs, and C3H/Wy hosts.



Fig 4 Section through liver of young (2 months) C3H f/Bi mouse which received 0 05 ml filtered leukemic tissue extract when newborn Note leukemic cell infiltration lymphocytic leukemia

Use of a supernatant fluid which was diluted with an amount estimated to be 2 1 (2 parts of supernatant fluid to 1 part of saline), after 7,000 g centrifugation, was followed by *only* parotid gland tumors and sarcomas—6 and 6, respectively—among 17 $C3H_f$/Gs injected and 1 sarcoma among C3H Gs mice injected. In some instances, parotid gland tumors and sarcomas occurred in the same individual, but neither appeared with leukemia. Tumors arising in C3H mice were transplantable to other C3H mice but not to the strains of extract origin—AKn, C58 and C57Br/cd At the present stage of development, these studies confirm the new and basic findings by Gross that preparations such as those mentioned have cancer-inducing properties and that the induced tumors are of the recipient rather than of the donor type.

## GRANULOCYTIC DISEASE INDUCED BY FILTERABLE AGENT

A second area of study involves a filterable agent causing a malignant disease of the hematopoietic system thought to be granulocytic leukemia[31] (Fig. 5). Normally, this virus-induced disease can be transmitted readily to adult individuals of the Swiss strain but not to adult individuals of certain other strains. Recently, we have been able to transfer this disease to the resistant C3Hf/Bi strain by means of inoculation of Selas porosity 03 filtrates into newborn C3H mice. Following this transfer, the disease has been passed to both adult C3Hf/Bi and adult Swiss mice by both cell and cell-free filtrate material.

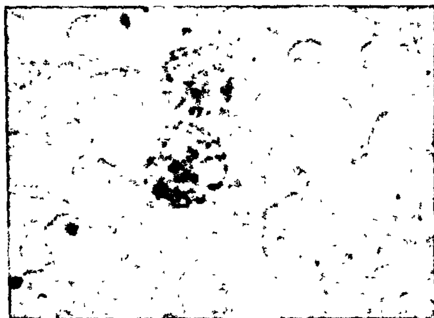


Fig 5.—Peroxidase reaction granules in stem cells in blood of Swiss mouse treated as adult with filtrate from spleen with "granulocytic leukemia"

## TREATMENT OF LEUKEMIC AND NONLEUKEMIC STRAINS WITH CORTISONE

Another study which relates to the problem mentioned concerns the occurrence of parotid gland tumors in cortisone-treated mice of both leukemic and nonleukemic strains. In our first studies,[32] we used strains AKR, C58, and a new strain developed by us following a cross between C58 and AKR Both C58 and AKR are high-leukemic strains which have been studied intensively by many investigators In our study, treatment consisted only of Cortone administered subcutaneously (1 mg. per day) for three successive days once each month throughout life, starting when the mice were 1 month of age Following this treatment, no parotid tumors originated in strain AKR, but they



Fig 6—Parotid gland tumor in cortisone-treated female mouse descended from original mating of C58 × AKR after inbreeding a few generations

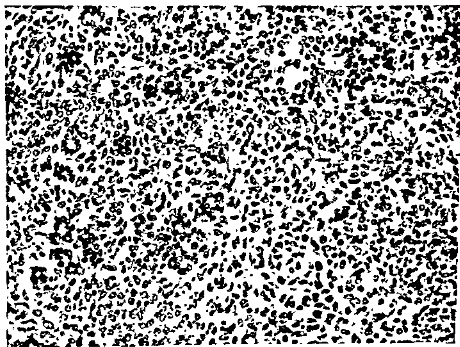


Fig 7 -Section through parotid tumor of C58 AKR cortisone-treated female mouse (See Fig 6)

did originate in male as well as in female mice of the other two strains A total of 6 were observed—2 in strain C58 and 4 in strain C58 × AKR, or in 3.5 per cent and 8 0 per cent of the experimental animals, respectively. (Figs. 6 and 7) None appeared in the control animals The average age at autopsy with this type of tumor was 11 months. The tumors were, in most instances, first observed at a somewhat younger age More recently, these rare parotid gland tumors have been observed in our Cortone-treated C3Hf/Gs mice, but not in C3Hf/Hu The results show that periodic treatment with Cortone has brought out a rare type of tumor in the mice mentioned, a type of tumor similar to that observed following treatment of newborn mice with leukemic tissue extracts and certain other cell-free preparations

## DISCUSSION

DR SYVERTON: The significant experimental findings summarized by Drs. Woolley and Gross do not eliminate for a virologist the possibility that Dr. Gross's three tumors originated from three distinct agents which I would like to believe are viruses. Use of tissue transfer or cortisone could evoke a small percentage of tumors for a carrier strain of mice. Certain strains undoubtedly are free of tumor viruses, others may maintain viruses in the carrier state When a mouse is made highly susceptible from genetic background and hormonal influence, tumors appear This sort of host-range response applies to all virus infections For example, the occurrence in mice of mammary tumors in from 1 to 5 per cent of a homozygous stock indicates to a virologist a latent or carrier infection with mouse mammary tumor virus X-irradiation, cortisone, tissue implantation, or almost anything can serve as a provocative agent. A most effective means for erasing genetic resistance and inducing receptivity is the purposeful use of cortisone and x-irradiation singly or in combination These comments are all basic questions for Dr Woolley and Dr. Gross to answer.

DR. WOOLLEY: Answering your last question first, the C3H/An (National Cancer Institute) subline, which seems to be strain resistant to leukemia, may be taken as an example Leukemia has occurred only a few times in this subline and only from extracts which we considered very potent. Perhaps with the addition of cortisone or x-irradiation an increased number of tumors will be found.

DR SYVERTON: That experiment should prove extremely interesting, particularly if cortisone or irradiation is employed We have learned, for example, that these two agents in combination can elicit evidence for a variety of infectious agents in hosts otherwise resistant.

DR BURDETTE: In such an experiment, how does one separate alteration of the mechanism of immunity, direct action on the virus, and possible carcinogenic properties of the agent itself, such as irradiation?



Fig 6.—Parotid gland tumor in cortisone-treated female mouse descended from original mating of C58 × AKR after inbreeding a few generations

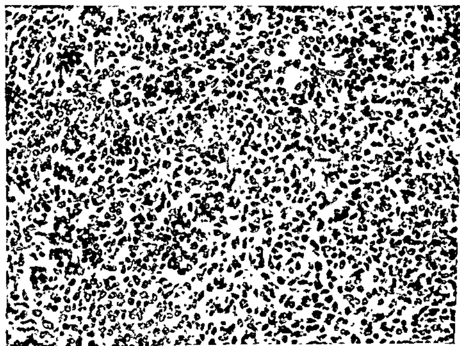


Fig 7.—Section through parotid tumor of C58 × AKR cortisone-treated female mouse (See Fig 6.)

DR. KIRSCHBAUM: May I ask one more question? Have you injected extracts of embryo from strains other than the C58 or AKR, and, if so, what has happened?

DR. GROSS: Nothing, other than the occasional appearance of parotid tumors.

DR. SPRAGUE: I would like to ask Dr. Woolley a question concerning his interpretation of results with animals treated with cortisone. If cortisone is an agent which facilitates viral multiplication and at times activates latent viruses, one could anticipate that leukemia may occur at an earlier time or perhaps in a higher percentage of such animals. Cortisone also has a striking lytic effect on lymphoid tissue, however, and perhaps this is the explanation of his results. I would appreciate his comments concerning this

DR. WOOLLEY: Cortisone is destructive and harmful to lymphoid tissue, whether it be normal or tumorous I would interpret the result as due to retarding or regressing influences on lymphoid tissue by cortisone. This is in contrast to findings for the parotid gland, where we know of no real effect of cortisone. Is that what you had in mind?

DR. SPRAGUE. Yes

DR. BURDETTE: Dr. Woolley, do you have any results to indicate that the maternal resistance factor is an agent similar to these viral agents under discussion, but with the opposite effect?

DR WOOLLEY. I have no results

DR. BURDETTE: Dr Syverton, would you comment on superinfection of viruses and competition between two separate particles of this type?

DR. SYVERTON. When invasion or infection of cells is limited to two viruses, both present in adequate numbers, competition must occur. Failure or prevention of infection by one of these viruses is known as interference. This capacity of one virus to act as an interfering agent for a second virus is a temporal relationship limited to days

DR BURDETTE: Dr Woolley, would you comment on the incidence of leukemia among mice with mammary tumors, and vice versa?

DR. WOOLLEY The results which I presented, showing that leukemia did not occur with mammary tumors, may be interpreted as competition between tumor-inciting agents, or on the basis that the sublines had been separated for a number of generations Among our inbred, tumor races, we do not have leukemia competing with breast cancer, or vice versa These diseases of mice seem to be well separated by nature

DR SYVERTON Is the inhibitor to which you refer labile and inactivated by heat?

DR. WOOLLEY Yes

DR. SYVERTON: These remarks are in continuation of the comments I just made. I assume that irradiation served as a provocative agent in contrast to the tumor-inciting agent or causative agent, virus or otherwise, already in the host. Activation can be effected by altering the host. For example, to cite our recent experiences, treatment of monkeys by use of irradiation and cortisone in combination revealed poliomyelitis virus in the vaccine responsible for the Cutter incident, while failure resulted from all other procedures for demonstration of polio virus.

DR. BURDETTE: In an ordinary type of viral infection, though, the result of the viral, infectious disease is not produced by either the cortisone or the irradiation

DR SYVERTON. I did not make myself clear. Treatment with cortisone and x-irradiation falls into two categories: (a) treatment to alter or abrogate natural host resistant to an infectious agent and (b) treatment for release of virus already present in cells. It would seem that the demonstration of tumor-provoking agent(s) in the C3H Bittner subline could be in this last category.

DR KIRSCHBAUM: Has leukemia appeared in C3H mice that have been injected with parotid tumor extracts from nonleukemic mice?

DR GROSS: No.

DR. WOOLLEY I have not had this experience either.

DR. KIRSCHBAUM: Has leukemia appeared in C3H mice that have been injected with normal, embryonic tissue of high-leukemic strains?

DR. GROSS. *These extracts were prepared from mice of leukemic strains*

DR. KIRSCHBAUM Leukemia has also appeared, according to the work of Graffi, in mice which have been injected at birth with extracts of the Ehrlich, ascites tumor Parotid tumors have appeared in C3H mice injected at 1 day of age with normal adult C3H tissue Now, the question I would like to raise is this Has leukemia appeared in C3H mice injected at 1 day of age with normal adult C3H tissue? Has that experiment been done? Furthermore, has leukemia appeared in C3H 1-day-old mice injected with normal nonleukemic tissue of other strains? That, I think, is an extremely critical control.

DR GROSS The only manifestation of neoplastic condition produced in C3H mice by injection of normal tissues was that of development of parotid tumor. Neither leukemia nor sarcoma was induced with normal tissues. However, we can induce a certain per cent of leukemia in C3H mice with x-irradiation.

DR. BURCHENAL Were you able to produce leukemia in young AKR mice from normal AKR embryos?

DR. GROSS That is something else again If one injects C3H normal tissues into newborn C3H mice, no leukemia results

DR. KIRSCHBAUM: May I ask one more question? Have you injected extracts of embryo from strains other than the C58 or AKR, and, if so, what has happened?

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DR. SYVERTON. Is the inhibitor to which you refer labile and inactivated by heat?

DR. WOOLLEY. Yes.

DR. WOOLLEY. We have no data on parotid tumors, but we, too, have used the C3H, cell-free, leukemia preparations in strain AK mice and have observed leukemia occurring at an early age (between 3 or 4 months).

DR. SYVERTON: This effect may be explained as dose and not superinfection in the sense it was used this morning. Use of newborn animals is a means for increasing the dosage effect, since animals before birth and within hours or days of birth do not respond as older or adult animals.

DR. BURDETTE. The viral etiology of leukemia has been emphasized in the preceding discussions, although Dr. Kirschbaum did review other factors known to influence the appearance of this type of neoplasm Since some information about the etiology of leukemia in humans is available, would the clinicians on the panel care to discuss how these results in animals may be carried over into the clinic? Dr. Schlosser, you may wish to discuss irradiation in relation to leukemia

DR. SCHLOSSER: This is a problem in which I am personally interested because it is recognized that the incidence of leukemia among radiologists and others exposed to low, intensive irradiation over long periods of time is higher than one would otherwise anticipate In addition, the same may be true for those exposed to radioactive isotopes

DR. ARROWSMITH: The appearance of leukemia a considerable time after irradiation of the thymus in childhood also suggests that this is a long-term, late effect of irradiation.

DR SCHLOSSER: I seem to recall a high incidence of carcinoma of the thyroid in children who had received radiation, although the dosage used for irradiation of the thymus is exceedingly small This may not be comparable to the irradiation received over a lifetime of handling radium and giving x-ray therapy.

DR BURCHENAL On the other hand, the thymus may be a particularly sensitive organ, especially at the age when the children are generally irradiated.

DR BURCHENAL Leukemias have been reported also.

DR BURDETTE. What is the relation of hormones to differences between the incidence and response of leukemia in childhood and later in life?

DR. BURCHENAL There is not much I can say about that. It is fairly well established that children under the age of 10 respond differently to agents, particularly folic acid antagonists, than children over that age, or adults Whether it has any definite bearing on hormonal changes or not is not known Adults over the age of 30 respond less well to standard agents than the group between 15 and 30.

Two other things regarding etiology could be mentioned. Bernard and his group believe that inhalation of benzol increases the incidence of leukemia Last, but perhaps not least, in our modern civilization it is not good from the point of view of leukemia to be within a thousand meters of the hypocenter of an atomic bomb when it explodes

DR. BURDETTE: Dr. Till, do you have any comment?

DR. TILL: Mention should be made here of the rare familial incidence of leukemia It is surprising to me that if a genetic factor is responsible, several siblings in a family are not more often affected with this disease

DR. WOOLLEY: I do not think that is too surprising. If one looks at the mouse population, for example, one finds that there are very few high-leukemia strains.

DR. ALFRED GELLHORN: *In man, certain leukemias have been causally* related to a variety of environmental factors Among radiologists and among many individuals exposed to the ionizing radiation of the atomic explosions in Japan, there is a high frequency of chronic myeloid leukemia It may be argued that radiation unmasks the latent leukemogenic virus, but it is odd, if this be true, that a chronic rather than an acute dyscrasia appears.

I should like to return to Dr. Till's remarks. It is true that an occasional case of congenital leukemia has been described, but this is a reportable rarity. It would be of interest to have the comments of those panel members who believe that a virus is important in leukemogenesis on the infrequency of leukemia in infants whose mothers develop the disease during the gestation period. It may be argued that the virus cannot pass the placental barrier However, one would still expect to see the disease in mother and child more frequently.

DR. KIRSCHBAUM. In a discussion of the relationship of the work on mice to the disease in humans, the report of Schwartz from Chicago should be included. He has obtained filtrates of brain from human cases of leukemia and inoculated them intracerebrally into mice of a high-leukemia strain and reported an accelerated onset of leukemia Similarly, brain extracts from mice have accelerated the onset of the disease in a high-leukemic stock He reports having passed the brain extracts through mice for three passages and then recovered the activity

DR. BURDETTE: Dr Sprague, did you have a comment?

DR. SPRAGUE I was interested in Dr. Burchenal's remarks concerning possible genetic factors Dr. Videbaek[53] conducted a large family study in Copenhagen, Denmark, and, as a result of this study, stated that the chance of developing leukemia by a member of a family where leukemia was present was 1 in 250 In a control group, the chance of such occurring was stated to be 1 in 4,000

DR. BURCHENAL. We have told our patients in the past that heredity played little role in leukemia, but in the past two weeks we have seen two families with two children affected with the disease This had not been encountered in the ten years previous

DR BURDETTE· Final remarks concerning etiology are now in order

DR. WOOLLEY: The results of studies on viral etiology give the promise of opening up new vistas and creating new problems not previously anticipated

Dr. Syverton: Our understanding of leukemia today is not unlike what was known twenty years ago of the rabbit papilloma-to-carcinoma sequence. Obviously, Dr. Gross and Dr. Woolley have made outstanding contributions in demonstrating that a transmissible agent is related to mouse leukemia. Since these agents provide opportunity for studies utilizing tissue or cell culture, it should be possible to arrive at three agents or a single agent, and, perhaps, to obtain each in sufficient quantity for characterization by the techniques employed for the virus of erythromyeloblastosis These findings are stimulating, since they indicate that agents or viruses may be shown to have causal relationship to mouse leukemia.

Dr. Kirschbaum: I think the information discussed is confusing as well as exciting.

*Arthur C. Upton, MD, and Jacob Furth, MD*

# FACTORS INFLUENCING INDUCTION *of* MYELOID LEUKEMIA *in* RF MICE *by* IRRADIATION*

We have been interested in the induction of myeloid leukemia in the RF mouse for some time Most reports of experimental work with induction of leukemia deal with lymphoid tumors or reticulum cell neoplasms Twenty years ago, however, an increased incidence of myeloid leukemia was noted in mice of the RF strain exposed to ionizing radiation [11] The present report summarizes the results of continued studies of some of the factors that influence the induction of this disease

The myeloid leukemias in the mouse[11, 14] and their differentiation from leukemoid reactions and extramedullary myelopoieses have been described in detail by Furth and Furth,[11] Upton and Furth,[14] and Barnes and Sisman [15] Most spontaneous myeloid leukemias in RF mice occur in animals 11 to 18 months of age, their onset is hastened in irradiated animals. There is extensive infiltration of the bone marrow, spleen, and liver, whereas the lymph nodes, thymus, and other organs are less affected Involvement of the peripheral blood is variable, as is the degree of maturation of the granulocytic series The disease, in general, is readily transplantable into isologous hosts, but some leukemias do not take in 100 per cent of the recipients, even on serial subpassages

*Work performed at Oak Ridge under Contract No W-7405-eng-26 for the United States Atomic Energy Commission

The thymic lymphomas of RF mice, as those in mice of most other strains, appear to arise in the thymus as lymphosarcomas. They may remain localized to the mediastinum, or they may become generally disseminated and thus involve the blood stream. The peak mortality from this disease occurs at the end of the first year of life in irradiated mice, and it is hastened in relation to the amount of radiation received.

In addition to myeloid leukemias and thymic lymphomas, other proliferative diseases of hemopoietic tissues occur in aging RF mice, but their incidence is not affected greatly by irradiation. Hence, they will not be discussed herein.

The results of two investigations are presented in Table VIII. In one, experimental animals received approximately 6 r of x-rays per minute and,

TABLE VIII

INCIDENCE OF MYELOID LEUKEMIA AND THYMIC LYMPHOMA IN RF MICE EXPOSED TO X-RAYS

| TYPE | LEUKEMIA INCIDENCE (PER CENT) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | MYELOID | | | | LYMPHOID THYMIC | | | |
| SEX | MALE | | FEMALE | | MALE | | FEMALE | |
| (R/MIN) | 6* | 60† | 6* | 60† | 6* | 60† | 6* | 60† |
| Total dose (r) | | | | | | | | |
| 0 | 4 | 1 | 0 | 0 | 6 | 4 | 14 | 19 |
| 128 | 19 | | 19 | | 9 | | 14 | |
| 350 | | 29 | | 18 | | 17 | | 33 |
| 512 | 11 | | 4 | | 17 | | 35 | |

*Data from Upton and associates[53]
†Data from Upton and Furth[54]

in the other, approximately 60 r per minute. We do not know whether this difference in intensity influenced the induction of leukemia, but, at the higher dose rate, x-rays have a somewhat greater killing effectiveness In the nonirradiated mice of both experiments, the incidence of myeloid leukemia was slightly higher in male animals, and a single dose of only 128 r increased the incidence several fold in both male and female animals A higher rate of induction was observed after 350 r, but, paradoxically, after 512 r the incidence of myeloid leukemia was lower than after either 128 or 350 r In contrast, the incidence of thymic lymphomas was higher in female animals, was not greatly elevated by exposure to 128 r, and increased progessively with the dose With this background, the present series of experiments was initiated

To explore the influence of sex hormones, we castrated the mice at 4 to 5 weeks of age, one week prior to irradiation (Table IX) The induction of myeloid leukemia, which was more common in male than in female mice, was inhibited by removal of the testes, but it was unaffected by removal of the ovaries Conversely, the development of thymic lymphomas was enhanced by orchiectomy and inhibited by ovariectomy

These observations suggest that in RF mice, as in mice of most other strains,[57, 59] estrogen is leukemogenic to the thymus The development of mye-

loid leukemia, on the other hand, apparently is not affected by estrogen but may be potentiated by androgenic hormone

The possible role of the thymus in the development of myeloid leukemia was investigated in a study of the effects of thymectomy. It was observed that removal of the thymus at 4 to 5 weeks of age, one week prior to irradiation, essentially abolished the induction of mediastinal lymphomas, as found previously by Kaplan[59] in C57BL mice, but it increased slightly the incidence of

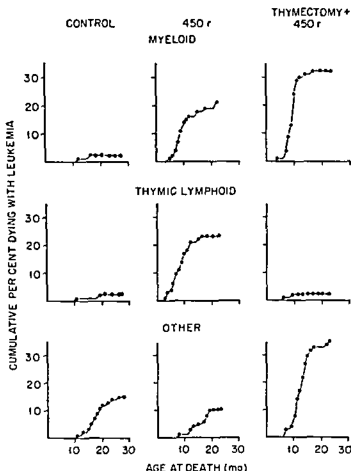



Fig 8 —Effects of thymectomy before irradiation on the induction of leukemia by x-rays in male mice of the RF strain

myeloid leukemia. In the absence of the thymus, an enhanced induction of lymphomas in extrathymic lymphoid tissues was noted also (Fig 8). These lymphomas tended to occur at the same age as the thymic lymphomas in intact mice and significantly earlier in life than the spontaneous nonthymic lymphomas in controls Thus, it appears that, in the absence of the thymus, other lymphoid

tissues manifest susceptibility to induction of leukemia. This has been observed also by Kirschbaum and Liebelt[60] in thymectomized mice treated with methylcholanthrene.

The slightly greater frequency of myeloid leukemias after thymectomy is presumed to result from reduction in the number of mice dying from thymic lymphosarcoma. It is noteworthy, however, that in thymectomized mice the incidence of myeloid leukemia and the combined incidence of myeloid and lymphoid leukemias were lower after exposure to 450 r than in intact mice exposed to 300 r (Table IX).

TABLE IX

THE INFLUENCE OF GONADAL FACTORS ON THE INDUCTION OF LEUKEMIA BY X-RAYS IN RF MICE

| DOSE (R)* | OTHER TREATMENT | LEUKEMIA INCIDENCE (PER CENT) MALE | | | FEMALE | | |
|---|---|---|---|---|---|---|---|
| | | MYELOID | LYMPHOID, THYMIC | COMBINED | MYELOID | LYMPHOID, THYMIC | COMBINED |
| 0 | | 1 | 2 | 3 | 3 | 17 | 20 |
| 0 | Gonadectomy | 2 | 9 | 11 | 2 | 12 | 14 |
| 300 | | 44 | 19 | 63 | 11 | 50 | 61 |
| 300 | Gonadectomy | 28 | 26 | 54 | 12 | 31 | 43 |

*In this experiment and those summarized in Tables X and XI mice were exposed in lots of 80 to 100 per treatment group with these factors of x-radiation 250 kvp, 30 ma TSD, 93 7 cm, 70-80 r/min in air with scatter, 3 mm Al filtration (Be window), hvl, 0 4 mm Cu

Because it was observed that shielding the thigh, that is, hemopoietic tissue in the bone marrow[61] or spleen,[62] during irradiation of the animal inhibited the induction of lymphoid leukemia, the influence of partial shielding on the production of myeloid leukemia was investigated. With the pelvis and lower extremities shielded by lead, mice were given a single dose of 450 r at 5 to 6 weeks of age, the shielded tissue receiving only about 2.5 per cent of the total dose The results indicate that the induction of myeloid as well as of lymphoid leukemia was inhibited out of all proportion to the amount of tissue shielded (Table X) If it is assumed that the pelvis and lower extremities constitute one-third of the entire body, the total dose of x-irradiation in gram-roentgens applied to only part of the body was much less leukemogenic than the same total dose administered to the whole body (Table X)

TABLE X

EFFECTS OF PARTIAL SHIELDING OF X-IRRADIATION ON LEUKEMIA INDUCTION IN RF MALE MICE

| DOSE (R) | INTEGRAL DOSE (G-R) | AREA EXPOSED | LEUKEMIA INCIDENCE (PER CENT) MYELOID | LYMPHOID, THYMIC | COMBINED |
|---|---|---|---|---|---|
| 0 | 0 | None | 1 | 2 | 3 |
| 150 | 3 000 | Whole body | 26 | 9 | 35 |
| 300 | 6,000 | Whole body | 44 | 19 | 63 |
| 450 | 9 000 | Whole body | 23 | 28 | 51 |
| 450 | 6 000 | Upper two-thirds body* | 8 | 7 | 15 |

*Pelvis and lower extremities shielded

The intravenous injection of nonirradiated marrow cells into totally irradiated mice has been observed to have a similar inhibitory effect on induction of lymphoma.[61] The mechanism whereby the presence of nonirradiated hemopoietic cells inhibits the induction of leukemia is not known. Transplantation studies suggest that the implanted, nonirradiated marrow cells survive, proliferate, and eventually replace those of the host that are destroyed by irradiation.[62-66] Kaplan[67] suggested that irradiation causes an imbalance between forces that promote the regenerative proliferation of hemopoietic cells and the capacity of the injured cells to respond. He postulated that it is primarily this imbalance and the resulting overstimulation by growth-promoting factors, rather than direct cellular effects of radiation alone, that initiate the neoplastic change. In support of this concept are the observations that neoplasia arises in nonirradiated thymic tissue implanted in previously irradiated thymectomized hosts.[68, 69] A mere imbalance, however, should not yield cells autonomous in normal hosts. A "mutagen" produced by irradiation must be postulated. Creation of regulatory imbalance and altered responsiveness of irradiated cells actually have been demonstrated in tumor induction by $I^{131}$.[70] It appears that some substance or substances present in normal marrow will minimize the likelihood of myeloid leukemia development after irradiation, but the mechanisms by which this protective power is exerted remain to be disclosed.

In another experiment, the influence of fractionation of the dose of radiation was investigated, since it had been reported that the lymphoma-inducing effectiveness of a given dose may be appreciably enhanced if the radiation is divided in appropriately timed fractions.[71] RF mice 5 to 6 weeks of age were given a total dose of 450 r of x-rays in three exposures, 150 r per treatment, with an interval either of two or of five days between exposures. The incidence of thymic lymphomas was significantly higher in the group exposed at intervals of five days than in those exposed at two-day intervals or in a single treatment (Table XI), thus confirming the observations of Kaplan and Brown.[71] In contrast, the rate of induction of myeloid leukemia declined as the interval between exposures was prolonged from two to five days. The mechanism of the observed enhancement of lymphoma induction by fractionation of the dose is unknown, but it has been suggested to be related to enhanced regenerative activity of

TABLE XI

EFFECTS OF FRACTIONATION OF X-IRRADIATION ON LEUKEMIA INDUCTION IN RF MALE MICE

| DOSE (R) | NUMBER OF EXPOSURES | INTERVAL BETWEEN EXPOSURES (DAYS) | LEUKEMIA INCIDENCE (PER CENT) MYELOID | LYMPHOID, THYMIC | COMBINED |
|---|---|---|---|---|---|
| 0 | 1 | 0 | 1 | 2 | 3 |
| 450 | 1 | 0 | 23 | 28 | 51 |
| 450* | 3 | 2 | 19 | 24 | 43 |
| 450* | 3 | 5 | 14 | 35 | 49 |

*450 r given in three doses 150 r per exposure

lymphoid tissues at the time of reirradiation. However, a morphologic and physiologic correlation between mitotic activity and leukemia induction is yet to be demonstrated. In general, the effectiveness of a given amount of irradiation decreases as the dose rate is reduced, as observed with the induction of myeloid leukemia under the conditions described.

SUMMARY

In the RF mouse, myeloid leukemia is induced by a dose of radiation lower than that required for lymphoma induction. Furthermore, the rate of induction of myeloid leukemia is maximal at levels of dosage below the lethal range and declines as the lethal level is approached, whereas the induction of lymphomas rises progressively with the dose

Myeloid leukemias predominate in male mice, and lymphomas predominate in female mice. Induction of myeloid leukemia is inhibited by orchiectomy before irradiation and is not affected by ovariectomy. The induction and spontaneous development of thymic lymphomas are increased by removal of the testes and reduced by removal of the ovaries.

Thymectomy before irradiation prevents the development of mediastinal lymphomas but enhances that of extrathymic lymphomas. Removal of the thymus also results in a slightly higher rate of induction of myeloid leukemia, probably by curtailing the mortality from thymic lymphoma earlier in life.

Shielding of the pelvis and lower extremities during irradiation of the animal inhibits the induction of myeloid as well as of lymphoid leukemia out of proportion to the amount of tissue shielded.

Fractionation of the irradiation may increase the rate of induction of thymic lymphoma but has not been observed to enhance the formation of myeloid leukemia.

## DISCUSSION

DR GROSS I am not familiar with the RF strain and wish to know more about it, particularly the incidence of spontaneous leukemia

DR. UPTON The RF strain was developed by Dr Furth in the 1920's at the Rockefeller Institute, and it has been inbred by him since then The spontaneous incidence of leukemia in the strain varies with sex. In males, the incidence of myeloid leukemia is generally around 3 per cent or less from experiment to experiment. The incidence of thymic lymphosarcoma (lymphoma arising in the thymus and becoming generalized to form lymphoid leukemia) is about 5 per cent or less In the female, the incidence of myeloid leukemia is likewise approximately 3 per cent, but the incidence of thymic lymphoid leukemia is between 10 and 15 per cent, or even slightly higher In RF mice, as in mice of many other strains, there are proliferative diseases of reticular and lymphoid tis-

sues occurring late in life. One is a granulomatous lesion arising predominantly in mesenteric lymph nodes, having some of the features of Hodgkin's disease. Dr Thelma Dunn[12] has called this disease "reticulum cell tumor, type B" In addition, there are other types of reticulum cell sarcomas, some of which may be referred to as monocytic leukemias We have endeavored to type most of these by histologic examination only. We feel this is a complex group of conditions, the incidence of which is not markedly affected by radiation. However, we have scarcely studied them and are not prepared to say very much about them.

As far as the transmissibility of the leukemias is concerned, we have attempted transmission of the myeloid leukemia in a few instances only, but the few cases tested have been found transmissible through successive passages In our experience, however, this disease has not produced 100 per cent takes in adult hosts We are hoping to obtain a transmissible myeloid leukemia in the RF mouse that may be useful in the study of factors influencing the growth of myeloid leukemia. However, we are discouraged after observing, with the several cases tested, that we cannot obtain more than 30 to 50 per cent takes in a single passage and that the use of newborn recipients and preirradiation of the recipients does not influence the outcome very much.

As for the method of irradiation, 300 r was given in a single exposure at a rate of about 80 r per minute, and the factors of irradiation were: 30 ma.; 3 mm. of aluminum, TSD of 93.7 cm , and hvl approximately 0 4 mm. of copper in the last experiments

In myeloid leukemia, the spleen is uniformly greatly enlarged and pale ordinarily This appearance of the spleen contrasts with that of the leukemoid state in terms of color, since the spleen in the leukemoid reaction is usually a dark purple or intensely red, whereas the spleen in myeloid leukemia is very pale, often even yellowish gray In some instances, there is slight enlargement of the lymph nodes, although usually not much Occasionally, when green infiltrations are seen, we refer to the disease as chloroleukemia The bone marrow is uniformly infiltrated, and very often there is massive necrosis of the femoral bone marrow.[24] The infiltrations are confined primarily to marrow, spleen, and liver without much involvement of lymph nodes

Maturation of the myeloid series in myeloid leukemias is variable Usually, the doughnut-shaped nucleus of the metamyelocyte is conspicuous in leukemic infiltrations and in the blood Also, elevation of the peripheral leukocyte count occurs ordinarily However, extensive studies on hematologic manifestations remain to be done in our laboratory

The thymic, lymphoid leukemia referred to in the present report is, we believe, a type widely studied in many strains of mice and similar to the radiation-induced leukemia in C57 Black mice investigated by Kaplan [13] The cells are usually lymphoblasts, occasionally, small cell lymphomas are observed We believe this disease arises in the thymus as a lymphosarcoma, widely infiltrates the

thymus and mediastinal structures, and may eventually become generalized and involve the blood stream, in which case we refer to it as lymphoid leukemia. Death in many instances results with mediastinal infiltration only, without any change in the peripheral blood.

DR. GROSS: How high was the mortality within two weeks after the 200 r and 300 r doses?

DR. UPTON: The $LD_{10}/30$ days in these mice would appear to be around 500 r. The animals in the present experiment were exposed at 5 to 6 weeks of age. We have not determined the $LD_{50}/30$ days accurately in the RF strain at this age, but at 10 weeks of age the $LD_{50}/30$ days is about 550 r. The mortality at the end of thirty days after 300 r is negligible

DR. KIRSCHBAUM: I am glad that Dr. Upton emphasized that there are varying mechanisms of induction of leukemia in mice. Kaplan has emphasized the role of the thymus, and we, too, have seen mesenteric lymphomas developing in thymectomized mice, indicating that extrathymic lymphoid tissue is susceptible to induction by x-rays, and this is also true for methylcholanthrene. If large enough doses of methylcholanthrene are used, the incidence of induced leukemia is as high in thymectomized as intact mice Concerning the shielding, did it make any difference which area of the body was shielded, and did shielding of lymphoid as well as myeloid tissue prevent the induction of granulocytic leukemia? Do you have any ideas concerning the effect of shielding on regeneration of the bone marrow and the effect of the rapidity of regeneration on the ultimate incidence of leukemia? Kaplan found by marrow shielding he had a more rapid regeneration of the thymus, and with a more rapid regeneration of the thymus, then leukemia did not occur

DR UPTON Dr Kirschbaum, these are questions we have asked ourselves and are at present attempting to answer As yet, we have no information about shielding structures other than the lower extremities and pelvis We have attempted to set up a more extensive shielding group, which is in progress, but we have no data to date We have not tried shielding lymphoid tissues as such to see whether this would influence the induction of myeloid leukemias It would be very desirable to know the rate at which hemopoietic cells recover following irradiation when hemopoietic parts are shielded Evidence in the literature suggests that when hemopoietic cells are shielded, they repopulate the irradiated hemopoietic tissues elsewhere in the body Observations by Lindsley and associates[64] with transplantation of homologous bone marrow indicate that, when nonirradiated marrow is put into an irradiated host (in this case marrow from donor rats having D-antigen on the red blood cells was put into recipient rats having C-antigen on the red cells), increasing numbers of D-marked red cells occur in the peripheral blood Similarly, Nowell and associates[66] found that rat bone marrow, when grafted into irradiated mice, produced circulating white blood

cells containing alkaline phosphatase in the recipient mice, whereas leukocytes in the mouse ordinarily were negative when tested for alkaline phosphatase. This evidence, added to that of the experiments of Lindsley and associates, supports the view that the nonirradiated marrow cells implant in the irradiated host, proliferate, and function hemopoietically. When part of the body is shielded, nonirradiated hemopoietic cells are probably enabled to survive and to seed irradiated tissues. However, this is extremely difficult to demonstrate conclusively.

DR. KIRSCHBAUM: What you say is very true concerning the seeding. However, Kaplan shielded bone marrow and still it was the thymus which regenerated rapidly.

DR. UPTON: *Further evidence regarding lymphoid regeneration has been reported recently from Britain by* Ford *and associates,*[41] *who have studied this question with marrow containing chromosomes* labeled with *translocations. They found that the tagged chromosomes specific to the implanted marrow occurred in lymphoid tissues in the irradiated recipient. From this they inferred, although their results are not fully reported, that the lymphoid elements in the irradiated host, as well as the hemopoietic elements of* the marrow, *were derived from the implanted marrow tissue* This is *a critical question and one that needs to have convincing evidence. I do not know whether this evidence is convincing, but it is strongly suggestive at least*

DR KIRSCHBAUM. Since gonadal secretion has such an influence on leukemogenesis, was any of the effect of radiation on the gonads, which might thus affect the induction of leukemia?

DR UPTON The gonads in the male were not exposed if they were lying in the scrotum during irradiation, because the entire pelvis was shielded In the female, it was doubtful whether the ovaries were included beneath the shield

DR WOOLLEY I am wondering if transplantation results with lymphoid leukemia were much different than with myeloid leukemia You mentioned results with the myeloid tumors

DR UPTON I regret to say we have studied the shielded tissues neither histologically nor from the hormonal standpoint Hence, I am unable to say what effect radiation may have had on the gonadal secretion We have made no study thus far of the transmissibility of the lymphoid tumors and have never attempted *to prove their biologic malignancy by transplantation*

DR WOOLLEY· Dr Sprague, how would these reticulum cell tumors behave with the Brachet test?

DR SPRAGUE We have had no experience with the Brachet test with tissues from patients with reticulum cell sarcoma We have had a single patient with replacement of the marrow with Ewing's sarcoma, and in this instance the cells behaved as mononuclear cells That is to say, the nuclei of these cells stained normally following ribonuclease hydrolysis I would like to ask Dr. Upton one

thymus and mediastinal structures, and may eventually become generalized and involve the blood stream, in which case we refer to it as lymphoid leukemia. Death in many instances results with mediastinal infiltration only, without any change in the peripheral blood.

DR. GROSS: How high was the mortality within two weeks after the 200 r and 300 r doses?

DR. UPTON: The $LD_{50}/30$ days in these mice would appear to be around 500 r. The animals in the present experiment were exposed at 5 to 6 weeks of age. We have not determined the $LD_{50}/30$ days accurately in the RF strain at this age, but at 10 weeks of age the $LD_{50}/30$ days is about 550 r. The mortality at the end of thirty days after 300 r is negligible.

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kemic cells no longer invaded the peripheral blood, and more and more primitive myeloid cells were seen in infiltrations of tissue on successive subpassages. Ultimately, the doughnut-shaped nucleus of the metamyelocyte became relatively rare, and that of the myeloblast occurred predominantly.

DR. GELLHORN: Does this persist, or do they die off?

DR. UPTON: I am not sure that I understand your question.

DR. GELLHORN: . . . if serial transplants are continued.

DR. UPTON: When we have done this, we have not obtained 100 per cent takes. We have had the unfortunate experience of losing two of our lines because the number of takes was small. Actually, because of our lack of success in obtaining uniform takes, we have almost abandoned attempts to study these in serial transplantation.

DR. KIRSCHBAUM: Before one concludes that leukemia is not transplantable, one must use a rather large number of animals in the first transfer. We have had the experience of taking a granulocytic leukemia as donor, and inoculating twenty mice, and having only one or two take. From the one or two transplants, lines may be established in which takes will occur in 100 per cent of the cases. I think some of these are leukemias of very low malignancy or very differentiated leukemias. Since they are differentiated, probably very few immature cells are in the inoculum, and the immature cells are the ones responsible for transplantation What the investigator may be doing is giving a very small dose of cells capable of transmitting the disease, and this dose is then increased by frequent transfer. Of course, one may be dealing with dependent leukemias, and the host must be conditioned before the inoculated cells can be grafted successfully

DR. UPTON: In the few cases in which we made serial subpassages, we did not see an increase in the number of takes although the cells appeared to be becoming progressively dedifferentiated From this we concluded that the neoplasm was conditioned in some way or that other factors were interfering with the uniform occurrence of takes

DR WOOLLEY: May I ask your method of transplantation how you prepared the tissue, how you inoculated, and whether it was subcutaneous or intraperitoneal

DR UPTON: The transfer of myeloid leukemia was made by dividing splenic tissue into fine pieces with dissecting scissors (The imprint of the spleen usually showed almost all myeloid cells) The splenic tissue was suspended in saline solution and injected intraperitoneally We tried intravenous injection as well, but from the results we did not feel that this was a more effective route

DR GELLHORN From the vantage point of one who knows nothing about the field and one who never expects to do any experiments in it, I would like to have you tell me what evidence exists that these really are leukemias Will you

question regarding shielding. Jacobson has shown that animals whose spleen was shielded could withstand higher doses of total-body irradiation. Is it necessary that active hematopoietic tissue be present in the shielded spleen for such protection to be afforded?

DR. UPTON: The spleen is a very mysterious organ to me. I believe, however, it has been the experience of those who have tried to promote recovery from radiation injury by shielding of the spleen that the technique yields best results in those animals which normally have hemopoietic activity in the spleen. Although there are differences between strains, mice usually have active myelo- and erythropoiesis in the red pulp. Hence, the shielding of the spleen under these conditions is, in fact, comparable to shielding the marrow. Furthermore, the embryonal spleen is characterized by active hemopoiesis, and much of Jacobson's protection work involved administration of embryonal tissues. In animals such as rabbits in which intrasplenic hemopoiesis does not occur, spleen shielding is not of much benefit.[74] Therefore, in answer to your question, the effect of spleen shielding probably amounts to shielding of hemopoietic tissue and is biologically equivalent to shielding of the marrow.

DR. SYVERTON. Have you tried transmission with cell-free extracts?

DR. UPTON We have attempted nothing of that nature. We are, of course, keenly interested in understanding what radiation is doing I, myself, have wondered if irradiation is unmasking a virus, let us say, and that we may have a lymphoid leukemogenic virus and possibly also a myeloid leukemogenic virus in the RF line A disease was discovered some years ago by Dr Furth in the RF strain which is characterized by the development of anemia, leukopenia, and erythropoietic splenomegaly[75] This condition was found to be transmissible with lyophilized material or cell-free material A virus or viral agents could exist in the RF mouse capable of producing a variety of disorders together or individually In our studies with filtrates we concentrated on newborn recipients, since the experiments of Dr Gross, along with the work of others, indicate that sensitivity is maximal in newborn animals

DR. GELLHORN What is the natural history of these leukemias? You say they do not transplant well. Do they destroy the host?

DR. UPTON I am embarrassed to admit that we know very little about the natural history of myeloid leukemia When animals die with this disease, they are extremely anemic. We have not performed extensive blood studies on them, but they are extremely pale, emaciated, and often exhibit massive necrosis of the femoral bone marrow The liver and spleen are usually extensively infiltrated by myeloid cells In the few attempts we have made to transmit the myeloid leukemias, the leukemias have undergone progressive dedifferentiation on successive passages In one of two cases in which the primary host had an elevated white cell count with many mature and immature granulocytic elements, finally the leu-

Charles C Sprague, MD

# DIAGNOSIS of LEUKEMIA

## INTRODUCTION

It is generally conceded that our current, conventional methods for diagnosis and classification of the leukemias are inadequate. Until recently, it was not necessary to make a precise, morphologic diagnosis in every instance. However, with the rapidly expanding use of chemotherapeutic agents, we have learned that the different leukemias vary greatly in their response to these agents. This makes it necessary that the best possible description be made in every case of leukemia One chemotherapeutic agent is presently available that is highly effective in a single type of leukemia, and yet totally ineffective in other types of leukemia Certainly, more examples of this will probably be seen as time goes on

The statement is made frequently that morphologists have had their day, and that little more will be achieved in this particular area of investigation I disagree with this viewpoint, and think that, with the application of new physical, chemical, and histochemical techniques, we will see additional valuable contributions made in this field Later in this discussion, I wish to summarize the results of our own experience with some of these techniques.

Initially, I would like to review briefly some of the methods that have been developed in the recent past, largely in research laboratories Granted that many of these techniques and methods are not suitable as routine procedures, some of them can be adapted with a minimum of difficulty. I shall try to make the discussion of these brief, confining my remarks to their usefulness and relative advantages and disadvantages

tell me why I am wrong in thinking if you destroy the bone marrow with x-ray or with drugs, you might not get the same sort of change that you have described here?

DR. UPTON: The evidence we have is that the animals all died of this disease, and, in the few attempts we have made to transmit it, we have been able to do so in some instances. The diagnosis was made at autopsy, and careful microscopic search did not disclose any other fatal process. There was widespread infiltration of primitive myeloid cells in the liver, spleen, and marrow, and foci of orderly maturation of granulocytes and erythrocytes were absent. This evidence causes us to view the disease as myeloid leukemia.

DR. KREMENTZ: Do RF mice develop other tumors after radiation?

DR. UPTON: The RF mouse will develop other neoplasms. We think there is a slightly increased incidence of pulmonary tumors in irradiated RF mice, but the major, neoplastic diseases are leukemias, lymphoid leukemia arising in the thymus and myeloid leukemia.

DR. KIRSCHBAUM: Dr. Gellhorn has raised a very pertinent question. Those of us who work on mice take pride in having a biological test, and this biological test is transplantation. In Dr. Upton's experiments, he has transmitted enough of these leukemias so he could assume those which do not transplant probably are leukemia. However, myeloid metaplasia may closely simulate leukemia, and if one found it sporadically in a strain, it might be very difficult to determine whether or not this was leukemia or hyperplastic reaction. We should emphasize transplantation as biologic evidence that this is truly leukemia. If one can transplant it, it is leukemia.

RIBONUCLEASE TEST

The ribonuclease test probably is unfamiliar to most investigators. Dr. Carrera, in our laboratory, is largely responsible for the work which I would like to report at this time. Brachet[78] first described the ribonuclease test in 1940. He showed that, following incubation in an aqueous solution of ribonuclease, the basophilic granules in the cell cytoplasm lost their ability to take up pyronine in tissue sections, whereas the staining of the chromatin of the cell nucleus by the methyl green component was unaffected. He, therefore, concluded that ribonucleic acid was located primarily in the basophilic, cytoplasmic granules. Using a modification of the method, Laves and Thoma[79] described the unique differential behavior which is shown by the nuclear chromatin of the white blood cells

This test can be performed in one of two ways. Methanol-fixed slides either of peripheral blood or of bone marrow can be hydrolyzed with a solution containing either ribonuclease or desoxyribonuclease activity. Ribonuclease, we find, is much more satisfactory for several reasons First, the hydrolysis can be carried out at 60° C., and the time is not critical. Fifteen minutes has proved satisfactory. Second, it has been found that freshly voided urine is an excellent source of ribonuclease. It is probable that there are factors in the urine other than ribonuclease responsible for its action on the cells Desoxyribonuclease, on the other hand, is an expensive enzyme, about six or seven hundred dollars a gram, and hydrolysis has to be carried out at 0° C for a period of fifteen seconds to one minute, the time being critical for optimal results

After hydrolyzing with one or the other of these enzymes and staining with Giemsa stain, the following results are observed with ribonuclease hydrolysis, the nuclear material of the neutrophilic granulocyte loses its ability to fix the stain, and it appears blanched The nuclei of eosinophilic granulocytes, lymphocytes, and monocytes, on the contrary, retain their ability to fix the Giemsa stain With desoxyribonuclease hydrolysis, the reverse situation prevails The nuclei of the mononuclear cells, monocytes, lymphocytes, and eosinophiles, are blanched, whereas the nuclei of the neutrophilic granulocytes stain normally

The findings are illustrated in the photomicrographs In Fig 9, a monoblast is seen which had been hydrolyzed with a solution containing desoxyribonuclease activity and then counterstained with Giemsa stain The nucleus of this cell is entirely blanched, the nucleolus and an Auer body are seen easily A neutrophil in the same field shows the nucleus to stain normally

Fig 10 reproduces a peripheral blood film from a patient with acute lymphocytic leukemia The smear was hydrolyzed with a solution containing desoxyribonuclease activity The nucleus of the lymphoblast is completely blanched, as was the monoblast in the previous illustration, and a neutrophilic granulocyte is included again for comparative purposes

## TECHNIQUES AND METHODS

### ELECTRON MICROSCOPY

In electron microscopy, examination of cell sections has proved much preferable to the casting technique. It has been found possible to section a granulocyte into some seven hundred slices, these slices being of the order of 0.002 micron thick. Such sections have proved very satisfactory for electron microscopy. The instrument, of course, provides tremendous magnification up to a range of 100,000 diameters; with the very thin sections, it was hoped that this would make possible better differentiation of the various blast cells and so-called stem cell, but, unfortunately, this has not proved to be the case. Mature cells can be distinguished without difficulty with this technique, but the immature blast forms still offer the same problem as previously.

Despite the potential usefulness of this technique, it offers two serious obstacles. First, the cells have to be examined in a desiccated state, and, second, it is necessary that the material be very thin, thinner than an intact cell. These two obstacles make it difficult, if not impossible, to examine intact cells. For these reasons, electron microscopy and micrography have been used chiefly in attempts to identify virus particles within cells, and in describing the submicroscopic structure of the cells.

There is at least one report where virus particles were supposedly identified in leukemic cells by this method. However, other investigators who have had extensive experience with this method have failed to confirm this observation.

### PHASE CONTRAST MICROSCOPY

This method allows one to examine live, unstained preparations providing a distinct image of the nuclear chromatin, nucleoli, nuclear membrane, mitochondria, specific granules, and Auer bodies. Those of you who have had an opportunity to visualize a vital preparation, using phase contrast microscopy, have been impressed undoubtedly with this as a technique. In addition to allowing visualization of these cells in a vital state, it has been possible to utilize a time-lapse movie technique, whereby the locomotion of the cells can be studied. It has been suggested that the actual locomotion of the cells may be important in terms of diagnosis. Such a movie utilizing this time-lapse photographic technique has demonstrated beautifully the development of the lupus erythematosus cell. The nucleoli and granules are easily identified, and both Jones and Moeschlin[illegible] pointed out the aid this provides in differentiating the blast cell of lymphosarcoma from the blast cell of lymphocytic leukemia. They stressed that the lymphosarcoma blast cell has a prominent single nucleolus, whereas the lymphocytic leukemia cell has two to four nucleoli. Brausil[illegible] stated that she has been able to differentiate a lymphoblast and a myeloblast, using phase contrast microscopy. Others have been unable to confirm her observations.

Fig. 11 is from a patient with acute granulocytic leukemia. In this instance, the slide was hydrolyzed with a solution containing "ribonuclease" activity (freshly voided urine). The nuclear material is blanched in all of the cells, as would be anticipated

The results of these studies suggest that in the case of the neutrophil and basophil, the nuclear protein was largely ribonucleoprotein, whereas in the mononuclear cells and in the eosinophil, it was desoxyribonucleoprotein This, I think, might explain in part the observation frequently made that eosinophils behave more like lymphocytes than basophils and neutrophils, as in the case of the lymphopenia and eosinopenia observed following the administration



Fig 11 —Appearance of smear from a patient with acute granulocytic leukemia after hydrolysis with a solution containing ribonuclease activity

of ACTH, for example This technique does not allow differentiation of the blast forms of lymphocytic and monocytic leukemia in that a lymphoblast and a monoblast behave the same way Fortunately, the differentiation of these two does not pose too difficult a problem as a rule I think you will agree that most of our difficulty is in differentiating monoblastic from myeloblastic leukemia, and it is here that the method seems to have its greatest usefulness.

## BIOCHEMISTRY AND METABOLISM OF LEUKOCYTES

Recent advances in the field of biochemistry and metabolism of leukocytes in the various leukemic states are of interest The level of amino acids has been found strikingly different in leukemic cells than in normal cells, this is



Fig 9—Monoblast hydrolyzed with a solution containing desoxyribonuclease activity and then counterstained with Giemsa stain



Fig 10—Film of peripheral blood taken from a patient with acute lymphatic leukemia and hydrolyzed with a solution containing desoxyribonuclease activity

The leukoerythroblastic peripheral blood picture that is observed in patients with metastatic carcinoma to the bone marrow can be confused easily with leukemia. The correct diagnosis usually is made at the time of bone marrow aspiration in such patients, although very commonly aspiration is impossible, and bone marrow section is required to provide the answer. The types of carcinoma most likely to produce this peripheral blood picture are carcinoma of the prostate, lung, and stomach. Multiple myeloma, on rare occasions, may be associated with such findings in the peripheral blood

Myeloid metaplasia commonly is misdiagnosed as leukemia, but, again, a careful evaluation of the peripheral blood, and bone marrow history and physical examination usually allow this to be differentiated from leukemia. Red blood cells in the peripheral blood of such patients show pronounced anisocytosis and poikilocytosis almost invariably. The marrow may be extremely variable, ranging from hypercellular to hypocellular. Biopsy of the liver or of the spleen is the most reliable method for establishing the diagnosis. Time will not permit a discussion of the interrelationship and some of the theoretical aspects concerning chronic granulocytic leukemia, polycythemia vera, myeloid metaplasia, and myelosclerosis. Probably they are closely related or actually variants of the same basic disorder.

## DISCUSSION

Dr Burdette Thank you, Dr Sprague, for revitalizing this morphologic subject and directing attention to pitfalls in diagnosis Discussion of Dr Sprague's paper is now open

Dr Woolley: I would like to know whether there is any point in the blast cell series when differentiation breaks down. I believe that certain blast cell leukemias do respond to cortisone, whether or not in the lymphoid or granulocytic series, and I wonder whether there is a point of embryonic differentiation or lack of differentiation where this difference is obliterated

Dr Sprague It is commonly recognized that there are some patients with acute leukemia where the cells are sufficiently immature to defy a morphologic diagnosis We have had occasion to study the blood and bone marrow of several such patients with the Brachet test and were able to classify them according to the behavior of the cells with ribonuclease hydrolysis. The subsequent course of the patient in each instance allowed us to confirm this initial impression

Dr. Gellhorn: Dr Sprague, first will you please comment on the report of Storti, from Italy, who recently described a simple test for differentiating between the various types of leukemia It consists of determining the osmotic fragility of the leukocytes of the buffy coat, using different concentrations of saline The leukocytic osmotic fragility is increased in acute and chronic

particularly true of the leukocytes in the acute leukemias. The ratio of ribonuclease to desoxyribonuclease is markedly elevated in acute leukemia, as is the ratio of uracil to thymine. Desoxyribonuclease inhibitor is found in large amounts in normal leukocytes, yet very little is in the leukocytes of chronic granulocytic leukemia; almost none is in chronic lymphocytic leukemic leukocytes and in the leukocytes of various acute leukemias. Apparently, there is a normal range of this desoxyribonuclease inhibitor in leukocytes found in patients with leukocytosis and leukemoid reactions

Valentine[80] found the alkaline phosphatase of cells in chronic leukemias to be less than normal, whereas in leukocytosis and in leukemoid reactions the level usually is normal or elevated Low values in leukemic cells do not return to normal even though a remission is induced Ellison[81] found the citrovorum factor content of leukocytes in acute leukemia to be elevated, whereas the level in cells of chronic leukemia usually is normal. Folic acid antagonist therapy markedly reduces the citrovorum level to values usually below normal

A great deal of work has been done on culturing bone marrow, and it is now possible to keep bone marrow as well as peripheral leukocytes viable long enough for short-term studies in vitro. With the use of radioactive isotopes, notably carbon 14-labeled formate or adenine, it has been possible to determine the rate of nucleoprotein synthesis, as well as to determine the effect of various chemotherapeutic agents on the rate of incorporation of one of these labeled precursors on the biosynthesis of nucleic acid

In view of our rapidly increasing knowledge of the chemistry and metabolism of these leukemic cells, it is not improbable that leukemias soon may be classified chemically as well as morphologically. Also, the technique of bone marrow culture may prove a very useful technique in screening leukemic patients for therapy It is possible that in time something comparable to current methods of determining the sensitivity of bacteria to various antibiotics can be offered

## DIFFERENTIAL DIAGNOSIS

Certain disorders may be confused with leukemia leukemoid reactions secondary to infections, particularly tuberculosis; collagen disorders, lymphomas, notably Hodgkin's disease; and liver disease, whether it be decompensated cirrhosis, metastatic cancer, or liver abscess, are at times extremely difficult to differentiate from leukemia As a rule, a careful examination of peripheral blood and bone marrow films, plus a thorough history and physical examination, will allow a differentiation to be made This can be extremely difficult at times, however; I can recall one instance where it was impossible to determine with certainty whether the patient had leukemia or leukemoid reaction, even following autopsy

factors present in urine, for they referred to the active principle as a "ribonuclease complex." Our results to date concerning the nature of the substance or substances in urine which account for the observed changes in the nucleus of neutrophilic granulocytes and its precursors may be summarized as follows:

1. Activity is not lost by dialyzing urine against tap water, but it is lost by dialyzing against distilled water.

2. The activity is slightly diminished after prolonged incubation at 58° C. at pH 7.7, and it is greatly diminished after heating to 100° C. for thirty minutes

3 The activity cannot be duplicated by commercial, crystallized ribonuclease, trypsin, or chymotrypsin.

4 The action can be closely simulated by sodium chloride solutions in concentrations ranging from 0.18 M. to 0 30 M and by phosphate buffer solutions of pH 7.7 of molarities between 0 06 and 0 08

5 The reaction of leukemic leukocytes appears similar to that of normal leukocytes

These results cast serious doubt on the hypothesis that the effect of urine is due to ribonuclease activity In fact, the only evidence suggesting any enzyme activity is the heat lability of the principle. Extraction of nucleoprotein by urine acting as a salt solution with the possibility of enzyme activity is now thought to be the most reasonable explanation of the reaction, although it has not been proved conclusively

lymphatic leukemia, decreased in acute myeloid leukemia, and similar to normal leukocytes in chronic myeloid leukemia.

Second, I would like to ask a rather impertinent question about the Brachet test. I had not known about this technique until reading your abstract, Dr. Sprague, and I was fascinated by it and very impressed by your report. Now, would you confirm once again for me, does it really work?

DR. SPRAGUE: In answer to Dr. Gellhorn's first question, I was unaware of Dr. Storti's fragility studies until the leukemia symposium in Detroit last month. We have had no experience with this method, but it certainly seems a simple method that may provide valuable information. We have been using the Brachet test for approximately one year and believe that it is quite reliable. There has not been a single instance in which it has misled us. The test undoubtedly requires some refinements, and I am sure there are pitfalls in the procedure. At the moment, however, we feel it is a very satisfactory laboratory method.

DR. KIRSCHBAUM: Are these really reflections of enzymatic activity? *With ribonuclease, one could expect ribonucleic acid to disappear. Here, one* has a complete washing out of the nuclear chromatin. Is this really enzymatic action?

DR. SPRAGUE: I also question whether it is, actually, because it is contrary to what one would anticipate from the known composition of nucleoprotein. We are not at all certain that the changes we observe in these cells are due to enzymatic activity of either ribonuclease or desoxyribonuclease alone. We are presently studying the effects of a purified enzyme to see how this compares with buffered urine as a source of ribonuclease activity. Although I am not prepared to say at the moment what accounts for the observed changes, this does not detract appreciably from the practical application of the method if further study confirms its worth.

DR. TILL: Did you perform the test more than once during the course of the disease in the same patient? Were the majority of tests performed when the diagnosis was first made?

DR. SPRAGUE: We have repeated the study on only one patient with chronic granulocytic leukemia. In this instance, there was no difference in the behavior of the cells before and following treatment with Myleran.

ADDENDUM

Since the above report, additional studies have been carried out in an attempt to elucidate the mechanism of the so-called "ribonuclease" test. In the initial studies, buffered urine was used as a source of "enzyme" as this had been reported by Laves and Thoma. They did not provide evidence of ribonuclease activity, however, and apparently believed that there were additional

basis for the discovery of compounds to use in the treatment of these dyscrasias Having justified the need for empiric screening of compounds in the treatment of leukemia, how is this accomplished? In the past few years, there has been a great deal of discussion on the optimal method or methods for antitumor screening. A cooperative experiment conducted by thirty-nine investigators in eight institutions has been addressed to the issue of what constitutes a reasonable biologic system for screening compounds which ultimately might be tested in the clinic against neoplastic disease. The over-all plan of the experiment was to test a standard group of compounds in as many diverse biologic systems as possible, correlating the results obtained with one another and with the effect of the compounds in human neoplastic disease

TABLE XII

| INSTITUTIONS | PARTICIPATING INVESTIGATORS |
|---|---|
| Bronx Botanical Garden, Bronx, N Y | Asheshov, I N<br>Flon, H<br>Hall, E A |
| Carnegie Institution, Cold Spring Harbor, N Y | Demerec, M<br>Hemmerly, J |
| Columbia University, New York, N Y | Davidson, J D<br>Freeman, B B<br>Gellhorn, A.<br>Golino, M<br>Hirschberg, E<br>Kells, A<br>Merson, G |
| Institute for Cancer Research, Philadelphia, Pa | Andre, J<br>Aronson, M M<br>Creech, H J<br>Hankwitz, R F, Jr<br>Hauschka, T S<br>Littleton, B J<br>Rothman N<br>Schultz, J |
| Sloan-Kettering Institute, New York, N Y | Clarke, D A<br>Karnofsky, D A<br>Philips, F S<br>Reilly, H C<br>Scholler, J<br>Stock, C C<br>Sugiura, K<br>Tarnowski, G. S |
| Southern Research Institute, Birmingham Ala | Schabel, F M, Jr<br>Skipper, H E<br>Thomson, J R |
| University of Minnesota, Minneapolis, Minn | Bittner, J J |
| University of Wisconsin, Madison Wis | Greenlees J L<br>Heidelberger, C<br>Keller, R A<br>LePage, G A |
| Wellcome Research Laboratories Tuckahoe, N Y | Bieber, S<br>Elion G B<br>Hitchings G H |

*Alfred Gellhorn, MD*

# LABORATORY *and* CLINICAL APPROACHES *to* LEUKEMIA CHEMOTHERAPY

## THE SELECTION OF COMPOUNDS FOR CLINICAL TRIAL

Unlike the effective methods which have been developed for the screening of potential antibacterial chemotherapeutic agents, the selection of compounds which merit clinical trial for the treatment of the leukemias poses a serious problem This is due to the fact that there is no curative drug which can be used as a standard against which to measure other compounds, and also because there is no biologic assay system which has been shown to be exactly comparable to human acute or chronic leukemia

How does one proceed? Ideally, we would like to have our colleagues in biochemistry and other basic disciplines provide sufficient information about the intimate characteristics of the leukemic cell so that it would be possible to develop chemical agents which would selectively destroy the abnormal cells A few weeks ago, it was my privilege to attend a conference at which a number of distinguished biochemists from this country and abroad discussed biochemical differences between normal and neoplastic cells After two days of vigorous discussion and examination of the evidence, it was agreed that there are a number of quantitative differences between neoplastic and normal cells, but that the fundamental biochemical characterization of abnormal growth still eludes definition

Since the day-to-day clinical problem of leukemia is so pressing, empiric methods of selecting drugs are in use, pending the development of a rational

if they proved effective as antitumor screens. Two bacterial mutation systems were included because of the possible relationship between mutagenic and antitumor action. The differentiation and developmental systems in slime mold, frog embryo, chick embryo, and the fruit fly, Drosophila, were studied as representing rapidly growing, normal tissues. The twenty-one biochemical indices of synthetic processes were included because of the possibility of more precise and sensitive indicators of antitumor action.

TABLE XIV

LIST OF COMPOUNDS

| COMPOUND NUMBER | NAME |
|---|---|
| 1 | [illegible] Mustargen) |
| 2 | [illegible] |
| 3 | [illegible] |
| 4 | [illegible] |
| 5 | [illegible] |
| 6 | [illegible] |
| 7 | [illegible] |
| 8 | Methyl carbamate |
| 9 | Chloramphenicol (Chloromycetin) |
| 10 | Potassium arsenite |
| 11 | 8-Azaguanine(5-amino-7-hydroxy-1H-triazolopyrimidine, Guanazolo) |
| 12 | 8-Azaxanthine |
| 13 | Benzimidazole |
| 14 | [illegible] |
| 15 | [illegible] |
| 16 | [illegible] |
| 17 | [illegible] |
| 18 | [illegible] |
| 19 | [illegible] araprim, Pyrimethamine) |
| 20 | [illegible] |
| 21 | D,L-Ethionine |
| 22 | β-2-Thienylalanine |
| 23 | Desoxypyridoxine |
| 24 | Colchicine |
| 26 | 5-Methoxytoluquinone |
| 27 | Netropsin |
| 28 | Azaserine (O-diazoacetyl-L-serine) |

Table XIV presents the twenty-seven compounds which the group of investigators selected for study. As can be seen, these included compounds which had been found to have activity against one or more of the experimental tumors, or to have strong activity against other biologic systems, as, for example, chloramphenicol, or were thought to be inactive, thereby serving as negative controls. The action of these twenty-seven compounds was studied in each of the systems previously described, and each investigator sent his result to a central office, where the information was collated. Eighteen hundred separate answers were obtained, an example of the flow sheet for two of the systems is shown in Tables XV and XVI. Table XV summarizes the effect of the compounds on the fifteen experimental tumors. The compound number is the same as given in Table XIV. Table XVI summarizes the effect of these compounds against the various microbiologic systems.

This experiment has been reported in detail elsewhere.[37] The participating investigators and their institutions are shown in Table XII. Table XIII lists the biologic systems which were used in the evaluation. The spectrum of fifteen tumors included two in the rat, one in the rabbit, ten solid and two ascites tumors in mice. Among the microbiologic systems studied, the five mouse-adapted viruses were included because of the oft-repeated postulation that compounds which would be effective against viruses would very likely be active against tumors, because of the central role of nucleic acids in these two systems. A number of bacteria and fungi were included because of the ease and rapidity of operating these systems, which would greatly facilitate screening.

TABLE XIII

BIOLOGIC SYSTEMS

| EXPERIMENTAL TUMORS | MICROBIOLOGIC | DIFFERENTIATION AND DEVELOPMENT | BIOCHEMICAL SYNTHESIS |
|---|---|---|---|
| Sarcoma 180 | Feline pneumonitis | Escherichia coli mutation SD4 | Tumor 755 DNA |
| Flexner-Jobling | Vaccinia | Escherichia coli mutation WP14 | Ehrlich protein |
| Walker 256 | Influenza A | Dictyostelium aggregation | Ehrlich adenine |
| Carcinoma E0771 | Western equine encephalitis | Dictyostelium culmination | Ehrlich guanine |
| Sarcoma T241 | Polio | Frog embryo differentiation | Gardner protein |
| Carcinoma 1025 | Bacteriophages | Frog embryo drug sensitivity | Gardner adenine |
| Mecca lymphosarcoma | Escherichia coli | Chick embryo abnormalities | Gardner guanine |
| Carcinoma RC | Bacillus subtilis | Chick embryo growth inhibition | Flexner-Jobling $Q_{O_2}$ |
| Recently isolated mammary carcinoma | Serratia marcescens | Drosophila larva | Flexner-Jobling $CO_2$ |
| | Staphylococcus aureus | Drosophila puparium | Flexner-Jobling protein in vitro |
| Carcinoma 755 | Mycobacterium smegmatis | Drosophila imago | Flexner-Jobling protein in vivo |
| Brown-Pearce carcinoma | Mycobacterium phlei | Drosophila larva/imago | Flexner-Jobling nucleic acids |
| Glioma 26 | Lactobacillus casei | Drosophila phenotype abnormalities | Flexner-Jobling adenine |
| Leukemia L1210 | Kloeckera brevis | | Flexner-Jobling guanine |
| Ehrlich ascites EF | Torulopsis utilis | Drosophila chromosome breakage | Spleen $Q_{O_2}$ |
| Ehrlich ascites ELD | Saccharomyces cerevisiae 358 | Drosophila crossing-over | Spleen CO |
| | Saccharomyces cerevisiae 376 | Drosophila variegation $W^{258\ 21}$ | Spleen protein in vitro |
| | Penicillium notatum | Drosophila variegation Ysc8 | Spleen protein in vivo |
| | Aspergillus fumigatus | | |
| | Streptomyces griseus | | |
| | Streptomyces antibioticus | | |

TABLE XVI

MICROBIOLOGIC SYSTEMS

| Compound number | Feline pneumonitis | Vaccinia | Influenza | Western equine encephalitis | Polio | Bacteriophages | Escherichia coli | Bacillus subtilis | Serratia marcescens | Staphylococcus aureus | Mycobacterium smegmatis | Mycobacterium phlei | Kloeckera brevis | Torulopsis utilis | Saccharomyces cerevisiae 358 | Saccharomyces cerevisiae 376 | Penicillium notatum | Aspergillus fumigatus | Streptomyces griseus | Streptomyces antibioticus | Lactobacillus casei |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | - | - | - | - | ++ | ± | ± | ± | + | ± | ± | + | + | + | + | ++ | ++ | + | ± | ++ |
| 2 | - | - | - | - | - | + | - | ++ | - | ± | + | ± | ± | - | ± | ± | + | ± | - | - | ++ |
| 3 | - | - | - | - | - | ± | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | - | - | - | - | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | - | - | - | - | - | | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - |
| 6 | - | - | - | - | | ± | - | - | - | - | ± | ± | - | - | - | - | - | - | + | ± | + |
| 7 | - | - | - | - | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 8 | Not done | Not done | Not done | Not done | Not done | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 9 | - | - | - | - | - | + | ++ | ++ | ++ | ++ | + | + | - | - | - | - | - | - | ++ | ++ | ++ |
| 10 | - | - | - | - | - | + | - | + | | + | + | ++ | + | + | + | + | - | ± | ± | ++ | ++ |
| 11 | - | - | - | - | | - | - | - | - | | - | - | - | - | - | - | ++ | ++ | - | ++ | ++ |
| 12 | - | Not done | - | - | - | - | - | - | - | - | - | | - | - | - | - | ± | - | ± | ± | ++ |
| 13 | - | - | - | - | | + | | ± | - | ± | ± | ± | - | - | - | - | + | ± | ++ | ++ | - |
| 14 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 15 | - | - | - | - | - | + | - | - | - | - | + | ± | + | ± | - | - | - | - | - | - | ++ |
| 16 | - | Not done | - | - | - | Not done | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 17 | - | - | - | - | - | + | - | ++ | - | ++ | + | + | - | - | - | - | - | - | ++ | ++ | ++ |
| 18 | - | - | - | - | - | ± | - | ± | - | ± | ++ | + | - | - | ± | - | - | - | ± | - | ++ |
| 19 | - | - | - | - | - | ± | - | + | | + | ++ | + | - | - | ± | - | - | ± | ± | ± | ++ |
| 20 | - | - | - | - | - | ++ | ± | ± | ± | + | + | + | - | ± | - | - | + | ± | ± | ± | + |
| 21 | - | - | - | - | - | - | - | - | - | ± | - | - | - | - | - | - | - | - | - | - | - |
| 22 | - | - | - | - | - | - | | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 23 | - | - | - | - | - | + | - | - | - | - | - | - | + | - | - | - | - | - | ± | - | - |
| 24 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 26 | - | - | - | - | - | ++ | ± | ++ | ± | + | ± | + | ± | ± | ± | ± | + | ± | ++ | ++ | ± |
| 27 | - | + | - | - | | ++ | + | ++ | + | + | ± | + | ++ | + | + | + | ++ | + | ++ | ++ | + |
| 28 | - | - | - | - | - | ± | ± | + | ± | - | - | - | + | ± | ± | ± | ++ | + | - | - | + |

TABLE XV

EXPERIMENTAL NEOPLASMS

| COMPOUND NUMBER | SARCOMA 180 | FLEXNER-JOBLING | WALKER 256 | MAMMARY CARCINOMA EO771 | SARCOMA T241 | CARCINOMA 1025 | MECCA LYMPHOSARCOMA | MAMMARY CARCINOMA RC | "SPONTANEOUS" MAMMARY CARCINOMA | MAMMARY CARCINOMA 755 | BROWN-PEARCE | GLIOMA 26 | LEUKEMIA L1210S | EHRLICH ASCITES EF | EHRLICH ASCITES ELD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | + | ++ | ++ | ± | − | ++ | − | − | + | ± | ± | − | ± | ++ | + |
| 2 | ++ | − | − | − | − | − | ++ | − | ± | − | − | − | + | + | ± |
| 3 | ± | ± | + | + | ± | + | ± | − | ++ | + | − | − | − | − | − |
| 4 | ++ | ± | ± | ± | − | + | ± | + | − | ++ | − | − | + | + | ± |
| 5 | − | − | ± | − | ± | + | + | − | − | + | + | − | − | − | − |
| 6 | + | ++ | + | ++ | ± | ++ | − | ++ | + | ++ | + | ++ | + | ± | − |
| 7 | + | + | + | + | − | ± | − | − | ± | + | − | ± | − | ± | − |
| 8 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 9 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 10 | − | − | − | − | − | − | − | − | ± | ± | ± | − | − | ± | ± |
| 11 | − | + | − | + | ± | ± | ± | − | ± | ++ | + | − | + | − | ± |
| 12 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 13 | − | − | − | ± | − | − | − | − | − | − | − | − | − | − | − |
| 14 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 15 | − | − | ± | ± | − | ± | − | − | − | − | − | − | − | ± | ± |
| 16 | − | − | − | + | − | + | − | − | ± | + | + | + | − | − | − |
| 17 | − | ± | − | ± | − | ± | − | − | ± | ± | − | − | − | ± | − |
| 18 | + | ± | + | ± | − | ± | ± | ± | ± | ++ | − | ± | − | − | − |
| 19 | + | − | − | − | − | − | − | − | − | − | − | − | ± | ± | − |
| 20 | ± | − | − | − | − | − | − | − | − | − | ± | − | ± | − | − |
| 21 | − | − | − | − | − | ± | ± | − | − | + | + | + | − | − | − |
| 22 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 23 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 24 | − | − | − | − | − | − | − | − | + | − | + | − | − | ++ | + |
| 26 | − | − | − | − | − | − | − | − | − | + | ± | − | − | − | − |
| 27 | − | − | ± | − | − | ± | + | − | ± | − | − | − | − | − | − |
| 28 | ++ | + | + | − | − | − | + | ± | ± | ++ | − | − | ± | − | − |

TABLE XVI
Microbiologic Systems

| Compound Number | Feline Pneumonitis | Vaccinia | Influenza | Western Equine Encephalitis | Polio | Bacteriophages | Escherichia coli | Bacillus subtilis | Serratia marcescens | Staphylococcus aureus | Mycobacterium smegmatis | Mycobacterium phlei | Kloeckera brevis | Torulopsis utilis | Saccharomyces cerevisiae 358 | Saccharomyces cerevisiae 376 | Penicillium notatum | Aspergillus fumigatus | Streptomyces griseus | Streptomyces antibioticus | Lactobacillus casei |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | − | − | − | − | | ++ | ± | ± | ± | ++ | ± | ± | + | + | + | + | ++ | ++ | + | ± | ++ |
| 2 | | | − | − | | + | − | ++ | − | ± | + | ± | ± | − | ± | ± | + | ± | − | − | ++ |
| 3 | − | − | − | | | ± | − | − | | − | | − | − | − | − | − | − | − | − | − | − |
| 4 | − | | − | | | | | − | | − | | − | − | − | − | − | − | − | − | − | − |
| 5 | − | | − | | | | | − | − | − | | − | − | − | − | − | + | − | − | − | − |
| 6 | − | − | − | | | ± | | | | − | ± | ± | | − | | − | − | − | + | ± | + |
| 7 | − | − | − | | | | | − | | | − | − | − | − | | | − | − | − | − | − |
| 8 | Not done | Not done | Not done | Not done | Not done | | | | | − | − | − | − | − | | | − | − | − | − | − |
| 9 | − | | − | | | + | ++ | ++ | ++ | ++ | + | + | − | − | − | − | − | − | ++ | ++ | ++ |
| 10 | − | | | | | + | | + | | + | + | ++ | + | + | + | + | − | + | ± | ++ | ++ |
| 11 | | − | − | | | | | | | | | | − | − | − | − | ++ | ++ | − | ++ | ++ |
| 12 | − | Not done | − | | | | | − | | | − | | − | − | − | − | ± | − | ± | ± | ++ |
| 13 | − | − | − | − | | + | | ± | | ± | ± | ± | − | − | | − | + | ± | ++ | ++ | − |
| 14 | − | − | | | | | − | | | | − | − | | | − | − | − | − | − | − | − |
| 15 | − | − | | | | + | | | | | + | + | + | ± | | − | | − | − | − | ++ |
| 16 | − | Not done | | | | Not done | | | | − | | − | − | − | − | − | | − | − | − | − |
| 17 | − | − | − | | | + | − | ++ | | ++ | + | + | − | − | − | − | | − | ++ | ++ | ++ |
| 18 | − | − | − | | | ± | | ± | | + | ++ | + | − | − | ± | − | − | − | ± | − | ++ |
| 19 | − | − | − | | | ± | | + | | + | ++ | + | − | − | ± | − | − | ± | ± | ± | ++ |
| 20 | − | − | − | | | ++ | ± | ± | ± | + | + | + | − | ± | − | − | + | ± | ± | ± | + |
| 21 | − | − | − | | | | | | | ± | − | | | − | | − | − | − | − | − | − |
| 22 | − | − | − | − | | | | − | | − | | − | | − | − | − | − | − | − | − | − |
| 23 | − | − | − | | | + | − | − | | − | − | − | + | − | − | − | − | − | ± | − | − |
| 24 | − | − | − | − | | − | − | − | | − | − | − | − | − | − | − | − | − | − | − | − |
| 26 | − | − | − | | | ++ | ± | ++ | ± | + | ± | + | ± | ± | ± | ± | + | ± | ++ | ++ | ± |
| 27 | − | + | − | − | | ++ | + | ++ | + | + | ± | + | ++ | + | + | + | ++ | + | ++ | ++ | + |
| 28 | − | − | − | − | − | ± | ± | + | ± | − | − | − | + | ± | ± | ± | ++ | + | − | − | + |

From the raw data obtained from all of the systems, a further condensation was made This is shown in Table XVII. The significance of this table can best be explained through an example. Compound No. 1 is nitrogen mustard

TABLE XVII

RESPONSE OF TUMOR AND NONTUMOR SYSTEMS TO THE AGENTS

| COMPOUND NUMBER | TUMOR SYSTEMS | | | | NONTUMOR SYSTEMS | | | |
|---|---|---|---|---|---|---|---|---|
| | + | – | PER CENT POSITIVE RESPONSE | BIOLOGIC ACTIVITY | + | – | PER CENT POSITIVE RESPONSE | BIOLOGIC ACTIVITY |
| 1 | 11 | 4 | 73 | + | 41 | 16 | 72 | + |
| 2 | 6 | 9 | 40 | + | 38 | 21 | 64 | + |
| 3 | 9 | 6 | 60 | + | 13 | 44 | 23 | – |
| 4 | 11 | 4 | 73 | + | 10 | 48 | 17 | – |
| 5 | 6 | 9 | 40 | + | 12 | 47 | 20 | – |
| 6 | 13 | 2 | 87 | + | 29 | 30 | 49 | + |
| 7 | 9 | 6 | 60 | + | 13 | 46 | 22 | – |
| 8 | 0 | 15 | 0 | – | 7 | 47 | 13 | – |
| 9 | 0 | 15 | 0 | – | 31 | 28 | 53 | + |
| 10 | 5 | 10 | 33 | + | 41 | 18 | 70 | + |
| 11 | 10 | 5 | 67 | + | 23 | 36 | 39 | + |
| 12 | 0 | 15 | 0 | – | 15 | 43 | 26 | – |
| 13 | 1 | 14 | 7 | – | 33 | 26 | 56 | + |
| 14 | 0 | 15 | 0 | – | 5 | 54 | 8 | – |
| 15 | 5 | 10 | 33 | + | 26 | 33 | 44 | + |
| 16 | 6 | 9 | 40 | + | 13 | 42 | 24 | – |
| 17 | 6 | 9 | 40 | + | 29 | 28 | 51 | + |
| 18 | 10 | 5 | 67 | + | 31 | 28 | 53 | + |
| 19 | 3 | 12 | 20 | – | 31 | 28 | 53 | + |
| 20 | 3 | 12 | 20 | – | 36 | 23 | 61 | + |
| 21 | 5 | 10 | 33 | + | 22 | 37 | 37 | + |
| 22 | 0 | 15 | 0 | – | 5 | 54 | 8 | – |
| 23 | 0 | 15 | 0 | – | 15 | 44 | 25 | – |
| 24 | 4 | 11 | 27 | – | 19 | 40 | 32 | – |
| 26 | 2 | 13 | 13 | – | 36 | 21 | 63 | + |
| 27 | 4 | 11 | 27 | – | 37 | 22 | 63 | + |
| 28 | 8 | 7 | 53 | + | 39 | 20 | 66 | + |

This compound produced a positive effect against eleven of the experimental tumors and no effect on four of the experimental tumors, or a percentage positive response of 73 The compound also produced a positive effect on forty-one of the nontumor systems and no effect on sixteen, or again a percentage positive response of 72 The results for each of the other compounds have been summarized in a similar manner It was decided quite arbitrarily that a positive biologic activity rating would be given to those compounds which affected one-third or more of the systems studied Thus, Compound No 1, with a rating of 73 per cent in both the tumor and nontumor systems, has a positive biologic activity rating. Having classified the compounds in this manner, it was possible to compare and contrast the effect of compounds on the tumor systems and on the nontumor systems. When this was done, it was found that the correlation between the action of a compound in the tumor systems and in the nontumor systems was poor, the over-all matching of activity between the two systems was no better than 50 per cent From this evidence, as well as a number of other analyses carried out, it was concluded that the action of com-

pounds against tumors was not merely an expression of general biologic activity, but rather that there was some specificity of action This general conclusion was further supported by the fact that no nontumor system was found to respond sufficiently like the tumor system to be used as a substitute Finally, an examination of the response of the individual tumors indicated that no single experimental tumor responded in such a way that it alone could be used as an antitumor screening system From the evidence available, it was concluded that several tumors should be utilized in such a screen.

A partial test of the validity of the conclusions reached in this experiment can be made by comparing the results obtained against the experimental tumors with the results obtained in the clinic. Table XVIII presents those compounds

TABLE XVIII

CORRELATION BETWEEN EFFECT OF "ACTIVE" COMPOUNDS AGAINST EXPERIMENTAL TUMOR SCREEN AND AGAINST HUMAN TUMORS

which were found to be positive in the experimental tumor screen, together with their effect against human neoplasms Table XIX summarizes this comparison for the compounds adjudged negative in the experimental tumor screen. Although not all of the compounds have been tested in the clinic, nor have the clinical trials been exhaustive, the correlation, on the whole, is encouraging

TABLE XIX

CORRELATION BETWEEN EFFECT OF "INACTIVE" COMPOUNDS AGAINST EXPERIMENTAL TUMOR SCREEN AND AGAINST HUMAN TUMORS

Many investigators think that drugs now used in the clinic are relatively ineffective and that, therefore, the screening system must be relatively faulty Before accepting this rather discouraging point of view, it should be noted that the effect of these drugs on the experimental tumors also leaves much to be desired. In fact, the action of these compounds on human neoplastic disease is, in the main, *greater than on the experimental tumors.* *It, therefore, would* appear to be reasonable to hope that when compounds are found which destroy experimental tumors effectively, their clinical activity will be comparably great.

## CHEMOTHERAPY IN THE CHRONIC AND ACUTE LEUKEMIAS

The clinical chemotherapy of the blood dyscrasias will be discussed at greater length and more authoritatively by Dr. Morwenna Till and Dr. Joseph H Burchenal, but I would merely like to offer our experience in the treatment of the chronic leukemias with two new drugs and also to present the treatment of acute leukemia in adults with massive doses of adrenal cortical steroids

The work of members of the Chester Beatty Institute in London, under the direction of Dr Alexander Haddow, in the development of new alkylating agents, has added two new compounds of interest. With Dr. George Timmis, *Haddow described the effect of 1,4-dimethanesulfonyloxybutane, Myleran, on* experimental tumors [82] From these observations, Drs D A. G Galton and M. Till carried out clinical studies which led to the use of this compound in the treatment of chronic myeloid leukemia.[83] I wish to mention our results only because they are comparable to those obtained by Galton and Till, but our dosage system is somewhat different from that used by the British investigators.[84]

In our series, we found that the daily oral administration of 10 mg. of Myleran can be used safely. *It is our practice to continue this dosage until* the peripheral white count has been reduced to 25 per cent of its initial level At this time, the drug is discontinued to determine the level to which it will fall without treatment The objective in all instances is to bring the leukocyte count to the normal range. Table XX summarizes our results, together with

TABLE XX

TREATMENT OF CHRONIC MYELOID LEUKEMIA WITH MYLERAN

| INVESTIGATORS | NUMBER OF CASES | DAILY DOSE (MG.) | REMISSIONS NUMBER | REMISSIONS PER CENT | DURATION REMISSION (MONTHS) |
|---|---|---|---|---|---|
| Galton and Till[83] | 30 | 4-10 | 26 | 86 | 0-48 |
| Petrakis and associates[85] | 11 | 2-25 | 7 | 64 | 0-19 |
| Haut and associates[86] | 16 | 4- 6 | 14 | 88 | 2-21 |
| Gigante and associates[87] | 14 | 10-20 | 12 | 86 | 0- 5 |
| Pribilla and Stollberg[88] | 10 | 2- 6 | 9 | 90 | 4-15 |
| | 81 | 2-25 | 68 | 83 | 0-48 |
| *Delafield-Presbyterian Group (1955)*[84] | 21 | 2-20 | 17 | 81 | 0-48 |
| Grand total | 102 | 2-25 | 85 | 83 | 0-48 |

those obtained at other clinics. In our estimation, which reflects the consensus, Myleran is a useful addition in the treatment of chronic myeloid leukemia

Bis-β-chlorethylaminophenylbutyric acid, also known as Chester Beatty 1348 and chlorambucil, recently was introduced into clinical therapy by Galton and his associates[89] at the Royal Marsden Hospital. Our experience with this compound[90] in the treatment of chronic lymphoid leukemia is presented in Table XXI. As can be seen, the results are less favorable than those obtained in the treatment of chronic myeloid leukemia with Myleran. This is not unexpected, for even radiotherapy does less well in the treatment of the chronic lymphoid dyscrasias than in the treatment of the myeloid variety.

TABLE XXI
RESULTS OF CB 1348 THERAPY

| DIAGNOSIS | NUMBER OF PATIENTS | OBJECTIVE IMPROVEMENT | | SLIGHT NONE | SUBJECTIVE IMPROVEMENT |
|---|---|---|---|---|---|
| | | EXCELLENT | GOOD | | MARKED OR MODERATE |
| Chronic lymphatic leukemia | 18* | 3 | 8 | 9 | 8 |

*Twenty courses

The treatment of acute leukemia in adults has lagged behind the treatment of this disease in children Recently, Hill and Vincent[91] reported that large doses of cortisone in combination with 6-mercaptopurine and other compounds could produce remissions in the adult acute leukemia. We have carried out an investigation over the past eighteen months at our clinic on the effect of massive doses of adrenal cortical steroid in adults with acute leukemia.[92] In eight of sixteen adults, hematologic remissions were achieved with doses of cortisone up to 6 Gm a day, or of prednisone in daily doses of 1 Gm In four of these eight, the remissions were complete with reversion of the bone marrow as well as the peripheral blood to normal. These results, together with trials in subacute leukemia and the blastic crisis of chronic myeloid leukemia, are summarized in Table XXII Although the remissions achieved were complete in a significant proportion of the cases, the duration was only measurable in months, and the longest duration of life achieved thus far has been one year The toxicity of massive, steroid dosage is appreciable, and it includes overwhelming

TABLE XXII
EFFECT OF PREDNISONE (1 000 MG DAILY) IN ACUTE LEUKEMIA OF ADULTS

| DIAGNOSIS | NUMBER OF CASES | HEMATOLOGIC REMISSIONS | | NO EFFECT | DURATION OF EFFECT (MONTHS) |
|---|---|---|---|---|---|
| | | PARTIAL | COMPLETE | | |
| Acute leukemia | 17 | 6 | 5 | 6 | 1-12 |
| Subacute leukemia | 3 | 1 | | 2 | 8 |
| Blastic crisis of chronic myeloid leukemia | 3 | 1 | | 2 | 2 |

infection, gastrointestinal ulceration and perforation, electrolyte disturbances, steroid diabetes, and psychic disturbances From the results of our study, we concluded that the demonstration that hematologic remissions could be produced in adult, acute leukemia by large doses of adrenal cortical steroids was of greater heuristic than practical value.

### THE EFFECT OF BLOOD DYSCRASIAS ON HOST METABOLISM

It is increasingly recognized that the destruction of the host by an invading tumor is less often due to impairment of vital functions through pressure or replacement of the infiltrating tumor cells than by more subtle and yet more devastating effects on the normal body economy. Fever, cachexia, and anemia constitute some of the manifestations of disruption of physiologic functions in patients with the leukemias. In the past few years, interesting and valuable insight has been cast on the mechanism of anemia in the blood dyscrasias[93] Marks and Bishop,[94] at our institution, demonstrated a fundamental disturbance in carbohydrate metabolism in patients with epithelial tumors and blood dyscrasias. This is characterized by a decrease in the rate of glucose removal from the circulation as determined by an intravenous glucose tolerance test. Of particular pertinence to the present discussion has been the demonstration that individuals with chronic leukemia who have been treated effectively with available chemotherapeutic agents show an improvement in the abnormal carbohydrate metabolism as well. The precise mechanisms involved in this change in carbohydrate metabolism are under study In this investigation, the chemotherapeutic agent is of great value as a research tool.

### SUMMARY

Problems involved in the selection of chemotherapeutic agents for the treatment of the leukocyte dyscrasias have been presented, together with methods of procedure currently employed. Three compounds which emerged from empirical antitumor screening and were applied to human leukemia therapy were presented briefly Finally, the use of chemotherapeutic agents as tools to study the pathologic physiology of leukemia was indicated

The vigorous investigative pursuit in these three areas demonstrates that research in the leukemias is proceeding actively and productively. It would appear that reasonable, if not spectacular, progress is being made

## DISCUSSION

DR. SEGALOFF Those of us who have followed the course of the experiments which Dr. Gellhorn outlined in the beginning have great admiration for the amount of work involved, and are disappointed at the conclusions that of necessity must be drawn from the data A continuation of screening proba-

bly will disclose a greater number of similar compounds, and perhaps some of the similar compounds will be somewhat better. Dr. Gellhorn, would you speculate as to the direction screening should take in an effort to obtain better, similar compounds? How would you select compounds for futher screening, and how would you select the dose of any given compound? It has been our experience in animal tumors that the largest tolerated dose is not always the optimum.

DR. GELLHORN: Dr. Segaloff has asked searching questions which could be the basis of extended discussion. With regard to developments in the area of experimental cancer chemotherapy screening, I am sure that Dr. Burchenal could comment on the usefulness of using mouse leukemias as test objects for the selection of drugs which may be effective in human blood dyscrasias. In our laboratory, we have attempted to develop a screening system which will aid in the discovery of drugs to inhibit the growth of, or destroy, human brain tumors. This system consists of a chemically induced glioblastoma multiforme in the mouse which can be serially transplanted in the strain from which it originally arose The tumor also is grown in tissue culture. Fragments of human brain tumors obtained at exploratory craniotomy are also grown in vitro. The effect of compounds is determined on the mouse glioblastoma in vivo and also in vitro and also on the human tumors in vitro. Ultimately, drugs which have an effect on some or all of the components of this multistrand screen are evaluated on patients with brain tumors. It is hoped that when sufficient data have been accumulated, this screen will be found to have relevance to a particular human tumor

Dr Arthur Kirschbaum has forcefully and persuasively presented the position that spontaneous tumors in experimental animals should be used for cancer chemotherapy screening since they may be more comparable to human cancer The pragmatic reasons for eschewing spontaneous animal neoplasms for routine screening are that they are difficult to obtain in the requisite number, their natural history is so varied that reasonable untreated control groups would be difficult to establish, and, further, no evidence has really been offered to indicate any real advantage of these tumors over the transplantable variety

The selection of the optimal dosage schedule is difficult Since it is not possible to translate laboratory results on this issue to the clinic, the procedure is necessarily empiric The initial objective is to administer a maximal tolerated dose If this is effective, it is gradually reduced until experience in many clinics leads to the definition of a dosage regimen with the optimal therapeutic index

DR KIRSCHBAUM After viewing this array of tests in a quest for something which will have antitumor action, it seems to me a little unlikely that such a thing will be found After all, the effect of estrogenic hormone on prostatic cancer is due to its physiologic action, and why would estrogenic hormone

have any effect on adenocarcinoma of the colon? One can easily understand why, in the case of prostatic cancer, there may be an effect, but not in the second instance. As *a matter of fact, anything which affects all tumors would* not be specifically antineoplastic, but its effect could be explained on some other basis. As far as transplanted and spontaneous tumors are concerned, what we would really like to do is test something which closely simulates the human disease. Because there are not enough spontaneous leukemias available at the present time is no reason to say that the transplanted disease is better merely because transplanted leukemias are more easily obtained. There is a very good reason why the transplanted leukemia is not a valid test medium. Treatment is instituted within one to four days after transplantation, and this interferes with histocompatibility. Once the leukemia is established, then there is no antileukemic effect, which strongly suggests that the drug is active in interfering with the establishment of the tumor. A correlation between the effect on the transplanted tumor and the effect on clinical disease is necessary *for a working basis*

If one uses spontaneous leukemias of mice, the results are as discouraging as they are in clinical leukemia. In other words, if the experiment is loaded so that results will be obtained, the investigator may be happy, but the patient is not any more improved. Finally, it is possible to obtain large numbers of spontaneous leukemias. We do not have a tremendously large colony, but we do have as many as six hundred spontaneous leukemias occurring in a year. I think that testing on spontaneous leukemia might very well precede testing on the human disease

I am not disparaging the effort with transplanted leukemias It serves to provide useful leads, but I think that we have to be realistic. The results are disproportionately encouraging, and I would question the statement that the effect on human leukemia is actually better than the effect of the corresponding *drugs on mouse leukemia* I *would like to know the measure of* effectiveness that is being used

DR. BURCHENAL: The important thing in a screening test in the first place is to have something that is moderately sensitive. If you screen, as we have, say 16,000 compounds in the past ten years, the important thing is not to miss one which might be effective against man That is one of the reasons we like sarcoma 180, because it seems to be a fairly sensitive test, and it is true we are not treating established sarcoma 180, but rather putting it in by trocar and starting to give the drug twenty-four hours after the tumor implant is inoculated That is not treatment. *That is a test for inhibition of growth.* and it is certainly true, as Dr Kirschbaum says, if one allows the sarcoma to be established for ninety-six hours, it is harder to treat. For preliminary screening, it does not matter too much whether bacteria or sarcoma 180 cells are screened, either in the growth inhibitory or the treatment stage The important

thing is to find something that indicates an effect. Then, later, after an effect is found, one can go on to something like established sarcoma 180, spontaneous leukemia, or established transplanted leukemia, and see what these much more rigorous tests reveal

We believe Dr Gellhorn has several discrepancies in his figures An example: Is methylformamide positive in mice and negative in patients? We have tried only five patients with this compound, and it is extremely toxic to the liver. It may be that if we could give twice the dose which we do give, it might have some effect on tumors As it is, we are limited by liver toxicity which is even greater in man than mice. Azaguanine causes marked dermatitis in man, which does not occur in the dog, and may be a specific sensitivity of human skin. Perhaps if we could give larger doses, we could affect leukemias or tumors. The colchicine derivatives, for instance, were down as questionable I remember initial positive work on colchicine in mouse leukemia by Dr Kirschbaum There is some question that it is active against Hodgkin's and other diseases, but it is pretty toxic. Some of the newer derivatives are positive. One of these newer agents, which is by far the most potent against sarcoma 180, is desacetylthiocolchicine It has an effect on chronic granulocytic leukemia The diaminopurine is effective in a fairly limited number of mouse tumors, and it is equally limited in its effect on leukemias In chronic granulocytic leukemia, it is possible to show an effect with this compound, but one must have a patient who is an iron man with copper-lined insides to do so, because it causes considerable nausea and vomiting If enough can be taken, it lowers the count We have seen that many times in the same patient

To come back to spontaneous leukemia, the only thing that would concern me about deciding on a trial in patients, on the basis of final tests against spontaneous leukemia, would be that there are probably different kinds of spontaneous leukemia biochemically, even in the same strain of mice A compound effective against one might not be effective against another, in patients as well as in mice I do think, however, that spontaneous mouse leukemia is closer to human leukemia than any of our transplanted leukemias

Dr Gellhorn. Both Drs Kirschbaum and Burchenal have stated that spontaneous mouse leukemia is more comparable to human leukemia than transplantable leukemia This appears reasonable, but only when the parameters are defined In the case of screening for antileukemic drugs, I know of no evidence which clearly demonstrates the superiority of the spontaneous form over the transplantable varieties Dr Kirschbaum says that he has six hundred animals with spontaneous leukemias per year—in good years This number of animals would make possible the screening of about six compounds, which is scarcely a sufficient annual output of work

Dr Burchenal There must be correlation with human leukemia, of course. The system is significant as long as it correlates with respect to response.

DR. GELLHORN: Dr. Burchenal makes a plea for the use of microbiologic systems in screening. I reiterate that, in the study discussed, fifteen separate species failed to indicate responses which correlated well with results for clinical or animal tumors. I hasten to add that microbiologic systems can, and have, given valuable information on mechanisms of drug action which, in turn, have led to the development of new antitumor drugs

Dr. Kirschbaum has asked for evidence that antitumor compounds are more effective in man than in animals This could be illustrated by many examples, but perhaps one will suffice. Nitrogen mustard can, and does, cause tumor regression in Hodgkin's disease and lymphosarcoma. Against experimental tumors, at best, it produces partial inhibition of the growth rate.

DR. SEGALOFF. How many compounds that are negative when screened have been effective against leukemia? The use of neither spontaneous nor transplanted mammary cancer in mice has been particularly helpful as an index for the most effective compounds for treating human breast cancer. Therefore, I wish to direct attention to possible screening of compounds which are not effective against tumors in animals but may be effective against human tumors

DR KIRSCHBAUM. I think that the correlation that Dr. Burchenal has demonstrated between transplanted leukemia and clinical leukemias is quite good, and I would be the last one to say that transplanted leukemia should not be used. I realize the number of spontaneous leukemias is too few for a routine program, and think spontaneous leukemias should be included The question arises as to why the transplanted tumor is not as comparable or not as good an index as the spontaneous tumor. One can immunize against the transplanted tumor, but not against the spontaneous tumor, and, following the regression of transplanted tumors, the animal is immune to reinoculation We know this host-immune response may contribute to regression, and this may not be part of the effect of the drug This is not true for the spontaneous neoplasm, and we have no evidence that the immune response in the patient contributes toward the regression

DR. GELLHORN That is impressive I could not question differences between the spontaneous and the transplantable animal tumor, but this is not the issue in our discussion The question is whether or not there is a peculiar value of the spontaneous versus the transplantable tumor in the selection of drugs which will be clinically useful

In answer to Dr Segaloff's comments, we do not have a great deal of evidence on the question of the potential hazards of false negatives in experimental chemotherapy screening. In the experiment which I have summarized for you, there were eleven compounds which had no effect on animal tumors Cross correlation with available clinical evidence indicates that the only com-

pound which might have been missed by the experimental tumor screen is colchicine. This drug did not produce an effect on the tumors, although it may, and note I say only may, have some effect on human tumors.

DR. SEGALOFF: Should you not test at least an equivalent number of compounds that are negative in your screening test against human leukemia?

DR. BURCHENAL: Another thing to keep in mind when talking of screening tests is that there are many screening tests. Most screening tests do not show the adrenal steroids to be especially effective against mouse leukemia. There are certain mouse leukemias that will respond to them, but there are not too many. Those might have been missed if only a couple of leukemias had been used. As to your answer, I do not see how we can tell, short of ten thousand compounds found negative for patients, and I do not know how we will do that.

DR. GELLHORN: We do not have time for ten thousand, but the eleven for the most part have been tested. This is my point.

DR. BURCHENAL: Colchicine is negative because of toxicity in man. There is nothing practical about colchicine. Dr. Kirschbaum uses it in his test against mouse leukemias.

DR. SEGALOFF: We are not talking about toxicity.

DR. BURCHENAL: I am talking about practicality.

DR. SEGALOFF: The question is whether it works or not, whether it is toxic or whether it is not toxic.

DR. BURCHENAL: If it is too toxic, only a relative effect is obtained. No matter what the toxicity, the compound can be useful if less than a toxic dose is effective, but if it is toxic at a dose which is ineffective, it is not useful.

DR. SEGALOFF: That is right. However, the liver can be irreparably damaged with a compound such as one already mentioned, but this really has no bearing on the point as to whether it is intrinsically an effective compound against leukemia itself. Admittedly, this limits whether or not one is going to use it in patients, but the fundamental question as to its effectiveness against leukemia per se has nothing to do with its toxicity.

DR. BURCHENAL: I do not agree with you. The whole idea of the screening program is to get a compound to be used in patients. I do not care how effective it is against leukemic cells if it cannot be used in patients because of toxicity. Do you see my point?

DR. SEGALOFF: We seem to have two different concepts. I believe that it may be possible to alter a compound so as to change its toxicity and retain its effectiveness against leukemia.

DR. GROSS: I think there is a fundamental difference between the transplanted leukemia and spontaneous leukemia, and, while it may be expedient

at the present time to use transplanted leukemia for the tests, it is possible that certain conclusions can be missed and certain effective compounds may be missed if only transplanted leukemias are used for the screening tests. Anyone who has worked with both transplanted and spontaneous leukemias knows there are basic differences In the case of spontaneous leukemia, after a suitable length of time, one has a variety of forms of leukemias, some chronic, some acute. These different forms also occur in human leukemia. Spontaneous leukemia can be compared to the virus-induced leukemia in the laboratory. At the present time, we have virus-induced leukemia in chickens which appears in two or three weeks in 100 per cent of the chickens inoculated with cell-free extracts. Why are chickens not used for the screening test? We may have, within a few years, a similar virus-induced leukemia as in mice. Perhaps not all, but selected compounds should be checked against spontaneous leukemia.

DR. TILL: We may not be wise to focus all our energies in looking for substances that are antimetabolites, substances that will destroy the leukemic cells. Perhaps whatever causes leukemia causes a deficiency of some essential substance within the cells, and, as a consequence of this, the cells are unable to mature.

DR. SPRAGUE. From the preceding discussion, it seems that there is no satisfactory screening method at the present time. It would seem desirable that the method of screening that would approach human leukemia as closely as possible would be the most desirable. Considerable work has been done in the field of bone marrow cultures, and, perhaps by improving such methods, tissue culture of human leukemic cells might prove the most satisfactory in terms of screening antileukemic agents Certainly, it would seem that a concerted effort along this line would be justified

DR BURCHENAL: Theoretically, that is an excellent idea I do not know the percentage of human leukemic cells that can be cultured, but my impression is that it is mighty low. Osgood has three lines of cells he has cultured, and is studying the use of synthetic media For the purpose of discussion, let us say the strains can be grown on synthetic medium and compounds can be tested against them The very fact that those three out of hundreds can grow in tissue culture must mean they are biochemically different from ordinary cells Until a method is available whereby we can culture every leukemic cell, I question the value of such a method, but it is something that should be pursued as an important technique If only special cells are cultured, they may be of limited value as far as the usual leukemic cell which is encountered is concerned.

DR. SYVERTON To date, we have failed to culture mouse leukemic cells, but it is not difficult to maintain them in vitro for twelve or more days Another approach is to employ, *in vitro, actively metabolizing cells in combination or*

admixture with a test antimetabolite or chemical and to add a virus to this admixture. Production of virus by the cell serves as an indicator of the ability of the test antimetabolite or chemical to influence the metabolism and/or kill the host cell In other words, the inherent capacity of the cell to produce virus is a more delicate measure of its metabolism than direct manometric measurement. This approach demonstrated that most so-called antiviral agents are anticellular metabolic agents which operate by inhibiting cellular metabolism and in turn by inhibiting formation of virus.

DR. BURCHENAL: Is it not true that normal human cells, growing in tissue culture, have changed and become neoplastic, so it is hard to say what a leukemic cell or any other cell will do in tissue culture?

DR. SYVERTON: I question the statement that human cells growing in tissue culture become neoplastic Supportive evidence consists of changes in anatomic or morphologic configuration, which can be attributed to influence of suboptimal growth medium, and to demonstration on transfer by injection of several million cells to man or animal in the production of a small nodule. This nodule shows on section cells at different tissue levels which competent pathologists interpreted as evidence of cancer This evidence is subject to other interpretation in so far as it applies to cells derived from cell cultures and not to spontaneous tumors I should appreciate Dr Burchenal correcting me if I misunderstand the evidence to date

DR. GELLHORN In closing, I would like to take issue with Dr Sprague's statement that our screening methods are inadequate At the present time, I believe we can only say that the compounds which have been screened are inadequate Until we have compounds which can cure mouse leukemia but fail to modify human leukemia significantly, we cannot indict the screening method I personally feel that when an effective drug against mouse leukemia is uncovered, it will also be of great value against human leukemia Let us hope that the time will soon come when the screening method can be put to critical test

*Joseph V. Schlosser, M.D.*

# IRRADIATION THERAPY *of* LEUKEMIA

Irradiation therapy is extremely useful in the management of leukemias, and it probably is responsible for more beneficial results than the majority of therapeutic chemical agents at hand. This does not mean that steroid or other chemotherapy is without value, or that they necessarily are competitive methods of treatment As a matter of fact, the two methods of treatment often are integrated. In certain situations, chemotherapy has allowed irradiation therapy which otherwise would not have been possible. Good examples for this management are found in cases of acute leukemia. In addition, chemotherapeutic agents offer additional treatment to curb a crisis, and then irradiation therapy may be given again For example, patients with leukopenia who have become resistant to further irradiation treatment have been given steroid therapy with a change in the white count, and then additional irradiation therapy has been administered In general, we prefer to start treatment with irradiation therapy *Perhaps, in a few years, we may say that we prefer to start with busulfan in* myelogenous leukemia

Certain differences exist between our approach to the treatment of lymphocytic and of myelogenous leukemia by irradiation Usually, an attempt is made to keep the blood count within fairly normal limits Most therapists attempt to do this whether treatment consists of irradiation or a chemotherapeutic agent However, we find that this is more important in the myelocytic group of leukemias than in the lymphocytic group In myelogenous leukemia, the blood count should be maintained as near the normal level as possible by irradiation, but, in lymphocytic leukemias, alteration of symptomatology is the more important. If the patient with lymphocytic leukemia has a tremen-

dous spleen, bulky lymph nodes, or painful areas, or if he is extremely weak or has anemia, irradiation is given until the findings and symptoms are corrected We have found that patients with lymphocytic leukemia can have elevated leukocyte counts for considerable periods of time, and still remain in excellent general condition with no particular complaints. On the other hand, in myelogenous leukemia, trouble usually follows an increase in the number of leukocytes. If the high count is allowed to persist, the patient usually exhibits more pronounced symptoms.

There are four major methods of x-ray therapy. The first is that most generally used, irradiation over the spleen The others are: local irradiation over either lymph nodes or visceral areas; the irradiation of large segments of the body, usually half of the trunk, either the chest or the abdomen, for extensive involvement in these areas; and total-body irradiation. There are some variations of these forms of x-ray therapy, but the small differences are of no consequence

Splenic irradiation probably is the one that is most frequently used, and usually we use it first, regardless of which type of case we are treating. Fields usually are 15 by 15 cm, with treatment alternating to the anterior and posterior spleen, starting at dosage levels of 25 r and increasing the dosage up to 100 r to 150 r per day. In unusual cases with bizarre skin and hemic manifestations, initial doses as small as 10 r over the spleen may be given, gradually increasing the dosage as before In the case of lymphocytic leukemia, irradiation over the spleen sometimes will also allow assessment of the sensitivity of involved lymph nodes to irradiation, since systemic effects may follow local irradiation over the spleen Sufficient regression in the size of peripheral lymph nodes may occur, so that much smaller doses need be administered to them subsequently In myelogenous leukemia, local irradiation over the spleen is continued if the response is good If it appears that excessive irradiation over the spleen is required for the desired response, then total-body irradiation is used.

Local x-irradiation, of course, is one of the most useful ways of administering this type of therapy. Even if the patient is having some type of chemotherapy, very frequently local zones of involvement will not regress successfully even in a patient who is showing a good general response to the drug, and residual zones of involvement may be left These can be in the form either of an enlarged spleen, enlarged local or mediastinal lymph nodes, or visceral involvement In many of these instances, doses in the range of about 1,000 r are delivered to the organ itself Often, complete disappearance of the masses occurs within one week Local lymph node involvement with lymphatic leukemia will regress completely with a similar dose in the same period of time.

Occasionally, patients present themselves with massive liver and splenic involvement, or with extensive pulmonary infiltration, and in this instance we employ a technique or modification of it that we have used for pulmonary metastases in other types of carcinoma. Very large fields, 25 by 25 cm. or 30 by 30 cm., are employed a distance of 150 cm. from the tube, and daily doses in air in the range of 50 r to 75 r are administered each day, alternating anterior and posterior ports. In the case of leukemia, the dosage required is lower than when carcinoma is treated. The usual dosage is 1,000 r to 1,250 r in air to the chest within a period of approximately one month. Leukemic infiltrations may disappear even sooner. Although a dose of 1,000 r in a month's time may seem smaller than 1,000 r delivered in one week to the small local areas of involvement mentioned previously, actually it represents higher energy absorption because of the tremendous volume of the field. In like fashion, when total-body irradiation is given, dosage is reduced even more than the usual 75 r per day tolerated by half of the trunk.

The last method of treatment is the one we are using more and more. As a matter of fact, some of our more recent patients have been treated by total-body irradiation almost exclusively, after a test dose of irradiation to the spleen to check the likelihood of a crisis with precipitous fall in the white count. We obtain blood counts at intervals of one month to six weeks, use total-body irradiation to bring the count to normal, and, when the count shows a tendency to rise, then give more whole-body irradiation. The method of Mayneord[95] is used in calculating the dosage of total-body irradiation. You will recall that when Dr. Upton wished to determine the energy absorption in his mice, he considered only the roentgens delivered and the weight of the mouse, because there is not appreciable absorption from point of entrance to exit. This distance in patients is sufficiently great to make a correction for the intensity of irradiation necessary Dosage is calculated on the basis of energy absorption per unit mass of tissue Our plan of treatment has been to administer daily doses of one megagram roentgen, alternating anterior and posterior exposures for four doses After a minimum interval of one day, this may be repeated for a maximum total of sixteen to twenty megagram roentgens.

We have compared the results obtained with whole-body irradiation therapy of twenty-seven patients with myelogenous leukemia to the survival of twenty-four patients treated with splenic irradiation When all factors such as survival for a period sufficiently long to receive total-body irradiation are taken into consideration, we found no significant difference between the survival following the two types of treatment, respectively Unfortunately, cases of lymphatic disease suitable for such a comparison were not great enough for valid evaluation

We have changed our views recently concerning irradiation for acute leukemia, due to the altered course of the disease following steroids and chemo-

therapy. Although we continue to be extremely cautious about the amounts of irradiation used, these patients frequently have enlarged lymphoid tissue in the pharnyx, tonsillar area, neck, and spleen which is symptomatic and may not only be very burdensome but also a factor in survival In one instance, lymphoid tissue in the pharynx was encroaching on the laryngeal and esophageal inlet. Small doses of radiation over these areas may produce remarkable regression, and the symptomatic improvement is worth while, even though temporary.

The remarks which have been made are based on an analysis of fifty-three cases of lymphatic leukemia and sixty-five cases of myelogenous leukemia treated by irradiation therapy. These data, as well as results obtained by others, indicate that survival of leukemic patients properly treated compares favorably to that following the onset of many types of cancer.

(For Discussion, see p. 118 )

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chronic myelogenous leukemia, busulfan is as effective as repeated splenic irradiation, and it is more convenient to the patient. We have not compared its efficiency with that of whole-body irradiation or radioactive phosphorus administered according to Osgood's recommendation, but we believe it to be superior to other chemotherapeutic agents such as triethylene melamine, urethan, benzene, or arsenic. The response is less erratic with busulfan, remissions are longer, and there are no side effects if the drug is administered properly. Patients have been maintained in good clinical and hematologic condition for periods of two to five years on busulfan alone.

Although various dose schedules were employed in the early stages of clinical trial, we have not used daily doses greater than 0.065 mg. per kilogram (4.0 mg. daily for most patients) in the last four years. An initial loading dose was used a few times but was not found helpful. Doses larger than 6.0 mg. daily offer no advantages and may cause severe bone marrow damage. These large doses cause the leukocyte count to fall as quickly as it does following splenic irradiation, but the subsequent rise in hemoglobin level is not greater, and does not occur sooner, than in patients receiving 4.0 mg. daily. In our experience, hemoglobin levels usually start to rise after four weeks of treatment and reach normal values after approximately three months. This pattern is similar in patients treated with either splenic irradiation or busulfan, and the rate of elevation in hemoglobin level depends upon the initial value, being independent of the rate of decrease in leukocytes. Patients receiving daily doses of 4.0 mg. busulfan normally show some fall of leukocyte count during the first month of treatment, but occasionally there is no significant fall for six or even eight weeks. This may lead to the abandonment of the treatment by some clinicians on the ground that the patient is resistant to busulfan. Patients showing this delayed response are often those who have received several previous courses of splenic irradiation with remissions of shorter and shorter duration between courses. These patients may do well on busulfan if it is administered continuously.

A small number of other patients who have received no previous radiotherapy may also respond slowly to busulfan. There usually is great subjective improvement, even though the leukocyte count falls slowly and the number of immature cells in the peripheral blood remains high. The spleen in such cases, although decreasing in size considerably, tends to remain moderately enlarged. We find continuous treatment is more satisfactory than intermittent treatment in these cases also.

Fig. 12 shows the slow response to busulfan in a patient who had received two previous courses of radiotherapy. The long interval before the leukocyte count reached normal levels should be noted; also, the hemoglobin continued to fall slowly during the first eight weeks of busulfan treatment, but it rose subse-

*Morvenna Till, MB*

# THE USE *of* CHLORAMBUCIL (CB 1348) *and* BUSULFAN (MYLERAN) *in the* TREATMENT *of* LEUKEMIA*

On this my first visit to the United States, it has been a great pleasure to meet so many distinguished workers in the field of leukemia, and to learn so *much about their ideas and opinions on the subject In return, I should like to* tell you something of the views of Dr. Galton and myself on the treatment of leukemia with two drugs first synthesized at the Chester Beatty Research Institute The first of these is busulfan (Myleran), and the second, γ-(p-di-2-chloroethylaminophenyl) butyric acid (chlorambucil), has the code name CB 1348 and is an aromatic nitrogen mustard suitable for oral administration

## TREATMENT WITH BUSULFAN

It is now widely accepted that busulfan can induce remissions associated with fall of leukocyte count, regression of the spleen, and a rise of hemoglobin in patients with chronic myelogenous leukemia The only patients we have seen who proved resistant to the drug initially were those already in blastic relapse, and one patient in whom there was no clear distinction from nonleukemic myelosis. We have found that in the long-term treatment of cases of

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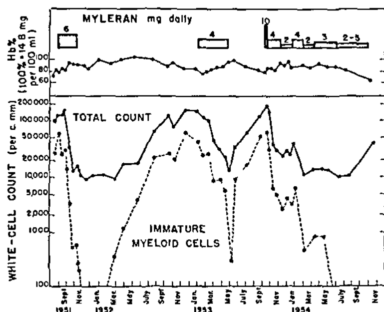



Fig 13—Blood counts of a patient with chronic myelogenous leukemia who received two courses of busulfan followed by continuous therapy (From Galton, D A G, and Till, M Lancet 1: 425, 1955)

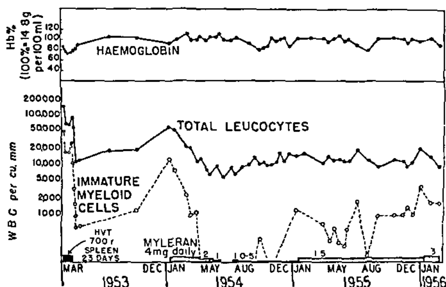



Fig 14—Blood counts of a patient with chronic myelogenous leukemia who received continuous therapy with busulfan following a single course of radiotherapy.

quently without the aid of transfusion. This patient remained well on continuous treatment with busulfan for more than two years During this time, he was very fit and able to carry on his business, which entailed extensive global traveling.

In new cases, we do not start treatment if the patient has no complaints. When symptoms occur, we give a course of busulfan, 4.0 mg. daily, and if, as in the majority of cases, the leukocytes fall satisfactorily, the drug is stopped when the leukocyte count reaches a level of approximately 10,000 per cubic millimeter. Such a course may last two to three months. After this, we do not treat again until symptoms recur, which may not be for another twelve months

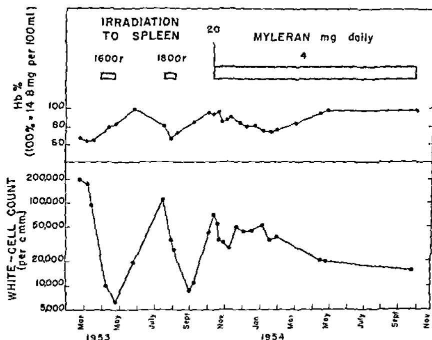



Fig 12 Blood counts of a patient with chronic myelogenous leukemia who was treated with continuous busulfan therapy after two courses of radiotherapy (From Galton, D. A G and Till, M *Lancet 1: 425 1955*)

or more Most patients show a slow but steady increase in total leukocyte count after the drug is stopped, and immature leukocytes reappear in the peripheral blood within six months Symptoms usually *do not recur until the hemoglobin* has fallen to 10 Gm , at which time the leukocyte count may be up to 200,000 per cubic millimeter. When busulfan is given again, the immediate response is as good as it was to the first course, but relapse starts earlier, and then continuous administration is required

The chart of a woman 40 years of age with chronic myelogenous leukemia appears in Fig. 13 The diagnosis was first made nine months before the period covered by this chart, at which time she received treatment with 3.5 mc $P^{32}$,

as continuous therapy for several months with twice that daily dose has been required in other cases. Similar variability of response to treatment in chronic lymphocytic leukemia is well known with other forms of treatment. We have treated only thirteen patients with chronic lymphocytic leukemia, and, of these, seven responded well. This is a small number of patients, and none has been followed for longer than three years; but our results suggest that the drug may prove useful in the managment of patients with this disease.

We do not treat symptomless patients, but, when symptoms occur, we start by using a dose of 0.1 mg. per kilogram (approximately 4.0 to 6.0 mg. in most cases) daily by mouth. This dose is increased to 0.2 mg. per kilogram daily after four weeks if there has been no response, but improvement is likely to occur during the third or fourth week of treatment. We discontinue the drug when the leukocyte count reaches normal levels or the lymph nodes and spleen have regressed satisfactorily. Treatment is started again only when symptoms recur. We feel that repeated short courses of chlorambucil are better than an attempt at maintenance. Continuous administration would risk bone marrow damage and possibly would give an illusion of maintenance to a remission that would have proceeded without further treatment.

Severely anemic patients may do well. Four patients with hemoglobin less than 6 Gm. had good remissions, with elevation of the value to approximately 12 Gm. within five to six months from the beginning of treatment, but neither of two patients with severe anemia and a positive direct Coombs' test derived benefit from the drug. There are no gastrointestinal side effects unless much larger doses are given intravenously. A most important toxic effect of chlorambucil, as with all cytotoxic agents used in the treatment of this disease, is the occurrence of purpura due to thrombocytopenia. Patients with clinical evidence of purpura are not suitable for treatment with this drug.

Initial thrombocytopenia, however, is not of itself a contraindication to the use of chlorambucil, provided the daily dose does not exceed 0.1 mg. per kilogram. Only one patient in our series developed purpura. Her platelet count initially was 45,000 per cubic millimeter, and, after four weeks' treatment with a dose of chlorambucil of 0.92 mg. per kilogram (twice the dose we now use), she developed widespread petechiae. There was no hemorrhage from mucous membranes. The drug was discontinued, and the platelet count subsequently rose to 20,000 per cubic millimeter. Remission continued for eighteen months. Patients with lymphocytic lymphoma but no peripheral lymphocytosis also vary in their sensitivity to chlorambucil. Those with heavy lymphocytic infiltration in the bone marrow require smaller doses than those with apparently normal bone marrow. Chlorambucil is easy to administer, and in our hands appears to be much safer than triethylene melamine. We feel that this drug is well worth further trial in the treatment of all types of chronic lymphocytic lymphoma.

The chart shows a satisfactory response to the first course of busulfan and a good initial response to a second course given fourteen months later. The second time, however, relapse occurred almost as soon as the drug was withdrawn, but maintenance therapy with busulfan effected control for a further twelve months. Blastic relapse occurred four years after the diagnosis was first made.

Patients vary greatly in the dose required during maintenance. Some are sensitive and require only 0.5 mg. daily to maintain the leukocyte count at normal levels. Others need 4.0 mg daily. Adjustments of 0.5 mg are made in the daily dose at intervals, according to the level of the leukocyte count. Usually, a patient requires increasing doses of busulfan as time passes, to keep pace with the progress of the disease Such increasing doses usually do not exceed the daily dose given at the start of treatment, and thus there is little risk of thrombocytopenia Fig 14 shows the chart of a woman 21 years of age who received continuous treatment with busulfan. The increasing requirement of the drug as time passes is well demonstrated. It is of interest that this patient became amenorrheic a few months after starting busulfan, and subsequently developed severe menopausal symptoms.

When resistance to busulfan eventually develops after long periods of maintenance therapy, this may manifest itself in three different ways There may be a relatively sudden rise in the peripheral leukocyte count, which consists mainly of blasts, there may be a fall in the leukocyte count to leukopenic levels, with a relative increase in blasts, or there may be a very slowly increasing anemia, to which the patient becomes well adapted, together with an enlarging spleen The peripheral leukocyte count may remain constantly between 20,000 and 30,000 per cubic millimeter in the latter group of patients, but the spleen may enlarge so as to fill the abdomen and give rise to symptoms due to pressure *The bone marrow in such cases usually is hypocellular* *If busulfan is discontinued*, the peripheral count rises very rapidly and contains a high proportion of primitive cells The patient experiences severe malaise, with high fever and generalized pains If another form of therapy is begun at this time, it should, therefore, be given in addition to busulfan, which may be withdrawn later when control has been secured with alternative therapy Radiotherapy to the enlarging spleen sometimes may relieve the discomfort for a short while, but it does not reduce the size of the spleen significantly All three clinical types of busulfan-resistant cases may respond for a short time to 6-mercaptopurine

TREATMENT WITH CHLORAMBUCIL

In marked contrast to the uniform response of patients *with chronic myelogenous leukemia* to busulfan, those with chronic lymphocytic leukemia show wide variation in their response to chlorambucil Remission lasting a year has followed treatment with only 0 1 mg per kilogram daily for three weeks, where-

*Joseph H Burchenal, MD*

# TREATMENT *of* LEUKEMIA*

### SCREENING OF AGENTS

We wish primarily to discuss the treatment of acute leukemias. Certainly, we need new agents, since none of the drugs that we have at the present time are too satisfactory in the treatment of leukemia. There are many ways of looking for these agents, and, although screening programs have already been discussed, I would like to mention the one used at the Sloan-Kettering Institute Compounds from various pharmaceutical houses, from chemical companies, and from departments of chemistry at various universities are assembled, their properties are catalogued, and a preliminary screening test with sarcoma 180 is used in mice In our estimation, this is the simplest technique and the one most economical of time and of mice Since 1946, over 16,000 crystalline compounds of known chemical structure and 10,000 additional unknown microbiologic filtrates have been included in these studies If they have an inhibitory effect or are promising otherwise, they may be tried in secondary tests.

Several strains of mouse leukemia (both those sensitive to and resistant to the conventional methods of therapy of human leukemia) and a spectrum of some twenty mouse and rat tumors are included in the screening Effects on the developing chick embryo also may be scrutinized carefully, since embryonic tissue often will respond to the same drugs that will affect cancer They also may be added to tissue culture in which normal and neoplastic cells are grown

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## REPRODUCTIVE SYSTEM AND THE THERAPY OF LEUKEMIA

Busulfan and chlorambucil, like other alkylating agents, cause extensive damage to the gonads of experimental animals. Similar damage in patients under treatment with either of these drugs is manifest by the occurrence of amenorrhea in women and gynecomastia in men. Mention was made earlier of our patient who became amenorrheic while on continuous treatment with busulfan. We have seen gynecomastia in two patients receiving busulfan and in one treated with chlorambucil. In all these cases, this condition was unilateral and lasted a few weeks only, in spite of the continued use of the drugs.

Pregnancy in leukemia is rare, but more than one author has described patients with chronic myelogenous leukemia surviving more than one pregnancy during the course of their disease There are various aspects to consider in the management of such cases.

The effect of the pregnancy may be very slight. Patients with acute leukemia tend to abort, but patients with chronic leukemia, in spite of their potential hemorrhagic hazards, tend to have normal pregnancies with no excessive bleeding at term, and the general course of the disease is unaffected. The effect of the mother's leukemia upon the child cannot easily be ascertained from the literature All reported infants were normal at birth. None was followed more than a few years In view of Dr. Gross's work, it would seem most important to know what happens to these children in later life.

It is the effect of the treatment of the leukemia upon the fetus that concerns us most today Small doses of splenic irradiation or radioactive phosphorus have been given to leukemic patients early in pregnancy without ill effects, and no patients so treated have required further therapy until after delivery. We, personally, have not treated any pregnant patients with busulfan, and we would hesitate to do so Bollag gave a single dose (10 mg per kilogram) of this drug to rats late in pregnancy, and he found the infant rats to be sterile, with atrophic gonads In view of this, it would seem unwise to give maintenance doses of busulfan during pregnancy Patients already receiving continuous treatment with busulfan are, however, unlikely to become pregnant, owing to the sterilizing effect of the drug

(For Discussion, see p. 118)

ually enlarge, or, toward the end of the disease, may develop bone pains These localized symptoms also may respond well to treatment by localized irradiation.

Patients who are not responsive to Amethopterin may respond to steroids, and the opposite is also true. Between the groups of the steroids, the folic acid antagonists, and 6-mercaptopurine, there is no cross resistance. Usually, however, if a patient is resistant to one steroid, he will be resistant to all steroids If he is resistant to one folic acid antagonist, all will be ineffective As to 6-mercaptopurine and thioguanine, if one has failed, the other probably will not be useful.

Steroids are useful in causing remissions in children with acute leukemia, quite useful in young adults up to the age of 30, and less useful over the age of 30, unless very high doses in the order of a gram of cortisone or Meticorten daily are used. Even with high doses, results in adults over the age of 40 are not nearly as satisfactory as they are in children. Folic acid antagonists act by conversion of folic acid to a reduced form, tetrahydropteroylglutamic acid, an intermediate in the production of biologically active folinic acid in the body These are the most useful of compounds in the acute leukemias of childhood Unfortunately, in our experience, over the age of 8 or 10 they are not as satisfactory as they are in younger children, and over the age of puberty they are effective infrequently. Using folic acid analogues alone, we have rarely seen remissions in adults I have seen a remission in one woman 28 years of age, and I have heard of one in a person 35 years of age, but they are extremely rare. Adults who have been treated with purine antagonists and either have had a remission and then developed resistance to it, or who have had treatment for a long period of time, may respond to Amethopterin. A trial, with cautious administration of the drug, seems justified in these patients

Mercaptopurine, thioguanine, and 6-chloropurine will produce remission in children and in adults with acute leukemia They also will produce remissions in patients with chronic granulocytic leukemia The effective dosage of these first two compounds in man is about the same We have not as yet seen any advantage of thioguanine over mercaptopurine, and we are not using it for that reason Also, 6-chloropurine in children seems to be about as effective as mercaptopurine, but no better Dr Ellison, in our group, is now studying 6-chloropurine in adults with acute leukemia, and there are some fragmentary preliminary data which seem to show that adults, particularly adults over the age of 40, treated with 6-chloropurine have a higher incidence of remissions Several cooperative groups are attempting to determine whether this is a real superiority, or whether it is due to a relatively larger dose of 6-chloropurine

Mercaptopurine is given orally, the usual dose is 2.5 mg per kilogram of body weight daily This amounts to approximately 50 mg per day in a child, and from 100 to 200 mg per day in an adult; it must be given for long periods of time Remissions under three to eight weeks should not be expected. This

side by side by the roller-tube technique. Compounds also may be tried for their effect against molds and fungi and bacteria. If a compound is effective against sarcoma 180, some of the mouse leukemias, and one or two other tests, clinical screening then is undertaken.

For clinicians, two things are of primary interest. (1) the maximum tolerated dose; (2) the system affected most. Dogs are used to investigate these questions, because organs damaged in the dog are likely to be the same ones affected first in the patient when he is treated with the drug Once data are obtained from canine experiments, cautious explorations with patients are possible. First, patients with far-advanced carcinoma are treated by increasing the drug gradually until beneficial effect is seen or the limit of toxicity is reached Calcium and uric acid excretion may aid in evaluating the effect of the agent used. Finally, the value of any new compound must be assessed in relation to other methods of treatment.

## PRINCIPLES OF TREATMENT

Certain basic principles that we are using in the treatment of acute leukemias may bear repetition. In the first place, we have two classes of agents that can be used in the treatment of acute leukemia. We have the antimetabolites, consisting of folic acid antagonists, purine antagonists, and, questionably, the glutamine antagonists The steroids comprise the other group Definite indications exist for the use of both types of agents. The steroids act more rapidly than the antimetabolites Improvement may occur within a week's time or even less when steroid is administered, whereas the antimetabolites rarely exert any beneficial effect on the bone marrow for three to eight weeks. The disease eventually becomes resistant to all these agents. This occurs more quickly with the steroids than with the antimetabolites as a rule, but steroids are useful at times when antimetabolites cannot be used On the other hand, when the patient's condition allows three to eight weeks for an attempt to obtain a remission, then the antimetabolites should be used and the steroids saved for the emergency, when time is at a premium. Conceivably, one might use the combination of steroids and antimetabolites, but we think it is better to reserve the steroids for the time which will surely come, when they and they alone will be effective. If the patient is acutely ill, has high fever, petechiae, and various evidences of bleeding, we think it is essential to start with steroids.

In addition to using these specific forms of therapy, one should use antibiotics at the first sign of infection Transfusions should be used when indicated, also Patients with meningeal infiltration may develop headache, papilledema, and other evidence of increased cerebrospinal fluid They usually can be benefited by irradiation over the skull, even though bone marrow and peripheral blood may not require treatment Also, some patients in fairly good remission, as far as marrow and peripheral blood are concerned, have kidneys which grad-

ually enlarge, or, toward the end of the disease, may develop bone pains These localized symptoms also may respond well to treatment by localized irradiation

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information has been circulated widely, but requests for advice from physicians indicate to us that it is not widely known. Although treatment lasts from six to twelve weeks as a rule, patients may at times become worse in a few weeks, and delaying steroid therapy obviously is inadvisable. In the absence of untoward symptoms, however, we continue treatment at least eight weeks before deciding it is ineffective. In some of the most satisfactory responses obtained, we have noted some evidence of remission in eight weeks, but not really a complete remission for ten or twelve weeks. The toxicity of the drug in this dosage level is minimal, but bone marrow depression occurs after excessive dosage When the leukemic count is high, the rapid fall in leukocyte count probably is therapeutic rather than toxic. In patients with a very high white count, particularly with extensive involvement of lymph nodes, a rapid fall in leukocyte count may be associated with abnormally high excretion of uric acid and with renal shutdown. In this group of patients, cautious administration is mandatory. Gastrointestinal complaints, including nausea and vomiting, may occur in adults given mercaptopurine, but they are rare. In contrast to the folic acid antagonists, which prevent the de novo synthesis of purines and pyrimidines, this compound interferes with the incorporation of preformed purines, their nucleosides and nucleotides, into nucleic acid

In general, the remissions are slightly shorter in a child with acute leukemia after treatment with mercaptopurine than those expected after treatment with folic acid antagonists Our series show approximately four months' actual remission as the average with mercaptopurine, exclusive of the time of treatment necessary to bring the patient into remission. The possibilities of combining it with other substances which might increase the length of remission will be discussed later

Mercaptopurine and other purine antagonists will produce remissions in a high percentage of the patients with chronic granulocytic leukemia. It is impossible to say at the present time how it compares with busulfan or irradiation because enough time has not elapsed for evaluation. The patient must be observed more closely to regulate dosage properly than when either busulfan or irradiation is administered In most cases of chronic granulocytic leukemia treated with mercaptopurine, one must treat the patient continuously, although occasionally a patient with this type of leukemia does well on intermittent therapy This is the exception rather than the rule

The compound o-diazoacetyl-L-serine has a definite effect against many animal tumors Unfortunately, results are not promising when it is given to patients It has a slight effect in terminal Hodgkin s disease, with responses lasting two to three weeks. In children with acute leukemia, it causes partial remissions, again lasting three or four weeks in an occasional patient In combination with quarter doses of mercaptopurine, however, quarter doses of diazoacetyl-L-serine in sarcoma 180 in mice have been shown by Clarke and associates[86, 87] to

be more effective than full doses of either compound alone For that reason, we have treated children with acute leukemia with this combination of azaserine and mercaptopurine. We begin with 2 5 mg. mercaptopurine per kilogram of body weight and an equal dose of azaserine. When the two compounds are given together, one is likely to encounter sore mouth, red tongue, and ulcerations of the mucosa of the tongue from the combination when given in this dosage. If this rather frequent complication occurs, the azaserine should be discontinued for a few days and then continued at one-half the original dose. Most patients will tolerate approximately 1 0 to 1.5 mg per kilogram of the azaserine along with full doses of mercaptopurine.

Nucleic acids are built in part from small units such as formate, glycine, glutamine, and $CO_2$, and, as the body gradually assembles various components of the purine ring, certain steps in this synthetic pathway may be blocked by various antitumor agents. The antifolic compounds, for instance, are known to block the addition of one-carbon fragment to glycine amide ribotide and are also known to block the incorporation of a one-carbon fragment into the 2 position, to make hypoxanthine ribotide. Mercaptopurine presumably blocks the conversion of a compound containing hypoxanthine into the adenine and guanine of nucleic acid. Azaserine blocks the conversion of formyl glycine amide ribotide to formyl glycine amidine ribotide Mercaptopurine and azaserine, in blocking the pathway at two different levels, should diminish the synthesis of certain nucleic acids necessary for leukemic cells, and thus have more effect than the one compound alone This principle of sequential inhibition was enunciated by Dr. Potter many years ago, and seems to have real possibilities in present-day chemotherapy For some reason, the combination of folic acid antagonists with mercaptopurine does not seem to be selectively synergistic in most tumor systems Slight synergistic effects are offset by additive toxicity

We studied a series of twenty-nine patients selected because they were not critically ill Of these twenty-nine patients, a surprisingly high number, twenty, had complete remissions as defined by criteria which have been promulgated by the Clinical Studies Panel of the Cancer Chemotherapy National Service Center Three patients had partial remissions, and six were failures More important, the average length of complete remission was 6 2 months Remission was tabulated independently from the total time of treatment Remission after combined treatment is two months longer on the average than with mercaptopurine alone Control studies are not satisfactory as yet in the group treated with mercaptopurine alone, and final conclusions must await a more comprehensive series For some reason, this combination of drugs has not yielded better results in our hands than mercaptopurine alone in adults with acute leukemia

Progress definitely is being made in the treatment of leukemia Tivey selected 208 cases of leukemia in children who had no specific therapy. They were treated at another time than those with which they may be compared, they

did not have comparable antibiotic and transfusion therapy, and many of the doctors who treated them did not have quite the zeal of modern chemotherapists. The median survival time in the series was 3.9 months from onset to death Among 154 children admitted to Memorial Hospital and treated with antifolic acids and/or the steroids, the median survival time was eight months In a series of 174 consecutive cases of acute leukemia in children treated since June, 1952, when mercaptopurine first became available to us in addition to the other agents, 50 per cent were alive over twelve months from the onset of their disease. It is not possible to report on the effect of mercaptopurine alone on survival time, since other therapy is instituted when relapse occurs. Some of these patients obviously were very ill on admission, were given a few doses of ACTH or cortisone, and died within a few hours or days after treatment was started They are all included in the series, however, since the other agents were available for their treatment if they had been indicated. These results and those of others indicate that progress, though slow, is being made in the treatment of acute leukemia compared to the results obtained ten years ago.

(For Discussion, see p 118.)

*William R Arrowsmith, MD*

# CLINICAL MANAGEMENT *of* LEUKEMIA

Some time ago, a well-known hematologist gave a talk on the use of urethan in the treatment of chronic myeloid leukemia and was quite enthusiastic. After the presentation, I had an opportunity to talk with him privately and asked him the kind of treatment he would request if he had chronic myeloid leukemia He said, "I would find the best radiologist I know and ask him to treat me." One of the things we have to keep in mind in a conference like this, or in reading about therapeutic agents, is the fact that, when we are concerned with screening and the effort to find out the fundamental processes, someone must study an entire series of patients on controlled or restricted methods of therapy On the other hand, if one has an individual patient who comes to him requesting treatment, no restriction as to the type of treatments from which to choose exists My remarks apply more to the latter situation

Even though comparisons of treated to untreated leukemia offer certain difficulties, there is no question about the prolongation of survival time and decreased morbidity in acute leukemia by means of current, therapeutic methods This is probably true also for chronic leukemia We must remember, however, that in addition to what we like to attribute to specific therapy, supportive therapy has improved and also influences survival time The widespread application of the Rh system and introduction of blood-banking which has enabled us to transfuse these people safely are two examples Antimicrobial drugs and steroids are additional examples

In the management of chronic leukemias, I have been influenced by an earlier interest in radioactive phosphorus and have depended on it more than any other single therapeutic measure This is, of course, a form of whole-body radiation The policy we have followed has been to try to obtain remission with phosphorus and then not to allow serious clinical relapse. This usually means more frequent treatment with smaller doses One of the impressions

obtained about the use of phosphorus in myeloid leukemia is that it will not reduce marked splenomegaly. However, if it does not respond to $P^{32}$, local irradiation is an effective supplemental treatment Likewise, radioactive phosphorus has been effective in the treatment of chronic leukocytic leukemia in those who do not have massive splenomegaly or adenopathy, and we have found it as satisfactory as anything else tried. Again, it can be supplemented at any time with local irradiation, nitrogen mustard, or triethylene melamine. Triethylene melamine requires cautious administration One advantage is that it may be given orally, although the possibility that it may be neutralized before absorption must be realized.

Since the patient with chronic meyeloid leukemia usually is symptomatic when seen, the trend toward treating patients with the disease at once is justified, particularly since the patient may not recognize the symptoms, and only after therapy does he realize the improvement that has occurred. Chronic lymphocytic leukemia is predominantly a disease of older patients. At times, it is difficult to know whether symptoms are due to leukemia or to some other condition. Also, the patient frequently will account for symptoms on the basis of age or something else familiar to him, and only after therapy does he realize symptoms were present. Therefore, the pronouncement that therapy should be given only when symptoms are present is not always easy to follow, and a therapeutic test may be required to decide whether symptoms are due to leukemia. Of course, even then the patient who is receiving therapy thinks he ought to feel better, and the effect of therapeutic trial may be difficult at times to evaluate.

One encounters complicating factors of hypersplenism in lymphocytic leukemia, particularly The possibility that really satisfactory control of a few of these patients may require splenectomy at some time during their illness should not be overlooked Purpural and hemolytic anemia are not at all uncommon, even when a patient appears to be well controlled. Splenectomy in leukemia was done fairly commonly in the earlier part of this century. It fell into disrepute, but now it is coming back into fashion In order to decide the difficult question of when to advise it, one must be sure that other measures to control the disease will not suffice and also that the general outlook of the patient is good enough to warrant major surgery

When the diagnosis of leukemia, particularly in its acute form, is made, a common reaction is that the patient should be admitted to the hospital and transfused. Dr. Burchenal has already indicated that neither is essential in the management of leukemia Certainly, one of the things we ought to do with these people, since all we have to offer them really is a modest prolongation of comfortable life, is to promote as normal and as happy a situation as we can for as long as possible. Youngsters are not at all happy about being removed from their home and placed in a hospital even when it is necessary. Likewise,

they are quite resistant to transfusions, and, while they may be anemic, most of these patients often are not having enough symptoms from the anemia itself to warrant hospitalization and/or transfusion. If therapy is at all satisfactory, clinical improvement will be rather prompt and will include a return of hemoglobin values to normal without transfusion; these measures, although they probably will be necessary later, should be avoided as long as possible.

Another problem which arises is the question of infections in leukemic patients and the advisability of antibiotics. I see many patients who have been started on antibiotic therapy the day the diagnosis was made and continued on some antibiotic throughout the entire course of the illness. This is particularly true in those patients with a low leukocyte count which may alarm the physician. Even if the white count is 100,000, almost none of these cells are of any help in defense against infection, and yet the patients usually come in without infections Prescribing antibiotics is adding to the expense and adds another hazard to therapy, since reactions may occur. If the patient or parent is an intelligent and responsible person who will recognize the indications for the use of antibiotics, then a prophylactic regimen can be avoided. If one does not have an intelligent patient, or parent, I think any type of program is more likely to be disappointing

I was happy to hear Dr. Burchenal point out that the outlook is not uniformly bad for adults with acute leukemia I have seen one man in the fifth decade of life who had remission for a year on folic acid antagonist. With the advent of 6-mercaptopurine, certainly enough adults have remissions to warrant a trial on therapy Earlier, I became discouraged with treatment of leukemia, particularly myeloid and monocytic, with the folic acid antagonists. Failure to respond and uniform toxicity make therapy seem inadvisable With 6-mercaptopurine, the incidence of remissions has been rewarding enough to warrant a trial in every adult and child who has the disease

In conclusion, I wish to comment on the attitude of the physician who cares for the patient. Too often, the patient is told or interprets the remarks of the doctor to mean that nothing really can be done This is deplorable enough in acute leukemia, but with the chronic leukemias it may lead to a long period, sometimes a year or two, of purposeless existence At a time when the patient can be free from symptoms on adequate therapy, he has them unnecessarily because of a defeatist attitude The withholding of therapy in patients with symptoms for use later in the course of the disease often is unwise Whatever the method of therapy chosen, and particularly in chronic leukemias, as much treatment as is necessary should be given at the beginning to produce as good a remission as possible or as long as possible by whatever amount of therapy is required, without worry about a future rainy day It will come eventually, but by that time the patient's ability to respond probably will be so diminished that the response will be disappointing, and he will have been deprived of a long

period of remission. Available methods of therapy should be exploited fully in management of the individual case, as well as in the optimistic search for improved methods and drugs. Certainly, the patient with leukemia can be treated today much more successfully than even a few short years ago.

## DISCUSSION

DR. BURCHENAL: I would like to continue Dr. Arrowsmith's discussion. I certainly agree that there is no point in keeping children alive if they are in the hospital and living an uncomfortable life. We find, on the average, 90 per cent of our children with acute leukemia are out-patients at any one time, and only 10 per cent are in-patients I would certainly also agree that they should not be transfused except when they have symptoms or when the hemoglobin suddenly drops to below 7.0 or 8.0 grams. We try to keep transfusions at a minimum, and usually we give transfusions on an out-patient basis. Antibiotics are used only when there are signs of infection, but they are then used quickly and in massive doses.

DR. TILL: Do you consider frequent bone marrow examinations necessary for satisfactory management of your patients, or are these part of an investigational program of the new agents under trial?

DR. BURCHENAL: We examine marrows more than is absolutely essential from the point of view of following the patient The habit arose because we were using intermittent therapy with folic acid antagonists. If marrows are examined every two weeks on such patients, signs of relapse in the marrow are seen when the clinical condition is excellent and the peripheral blood shows no sign of relapse Therefore, one is able to resume therapy and obtain remission as far as the marrow is concerned before there has even been clinical relapse From the research point of view, it is important for detection of remission from compounds tested, and it is part of our protocol to do them in the out-patient department every two weeks in all children and in most adults every week when they are in the hospital under treatment On the other hand, they are not necessary that often if a new drug is not being studied

DR SPRAGUE. There are three questions that I would like to ask Dr Till First, what is known regarding the pharmacologic action of busulfan? Second, have you treated any group of patients with maintenance therapy, and, if so, what were the results achieved? Third, do you feel that in the busulfan-treated group there has been an increase in the incidence of blastic crises, and, in such patients, what do you feel is the optimal form of treatment?

DR. TILL: Practically nothing is known of the pharmacology of busulfan Chemically, it is an alkylating agent as is nitrogen mustard, but the effects produced by busulfan upon the hemopoietic system of the rat are very different from those seen following comparable doses of nitrogen mustards. Studies are being planned with $C^{14}$-labeled busulfan, but I cannot give any data on this at present

In answer to the second question on the use of maintenance therapy, I do not know of any series of patients given busulfan continuously from the start of therapy. We have eight patients so treated; four of these received continuous treatment because they were x-ray resistant and two because their initial response to busulfan was slow. The other two patients received continuous therapy as a trial. One of these has done well for more than two years after the start of therapy. The other lived two years after the diagnosis was first made, but after the first year her hemoglobin began to fall, in spite of continuous reductions of the dose of busulfan, she slowly became leukopenic. When the drug was eventually stopped, the leukocyte count rose to normal levels but showed a substantial proportion of blasts. Her anemia persisted, treatment with 6-mercaptopurine was not helpful, and she required frequent transfusions during the last six months of her life. This case history would suggest that it is unwise to administer busulfan continuously from the time of diagnosis as a routine.

In answer to the question regarding the incidence of blastic crisis in busulfan-treated patients, I do not think the incidence differs from that in patients treated by other methods. Patients in blastic relapse do not respond favorably to increase in dose of busulfan, but a minority will respond for a while to 6-mercaptopurine.

Dr. Arrowsmith: Would you comment on morphologic findings in blast cell crisis?

Dr. Till: Our specimens were obtained by aspiration, not trephine biopsy. In every case in which marrow puncture was performed at this stage of the disease, marrow was obtained only with difficulty and fragments were few. Microscopically, there was marked hypoplasia, the commonest cells being bizarre megakaryocytes consisting of a single small nuclear fragment surrounded by platelets.

Dr. Kirschbaum: Dr Arrowsmith mentioned the improved methods of transfusion administration and the use of antibiotics. To what extent can increased survival be attributed to specific antileukemic therapy and to what extent to better medical management?

Dr. Burchenal: Unfortunately, as you know, there is no really good series which is parallel in time to these more recent series since 1953, and treated with none of the specific agents. The only figures I can give you are those from a small series of nineteen cases treated between 1946 and 1948 with the mustards, which are fairly nonspecific agents in acute leukemia. They had the transfusions and some of the antibiotics that are available today, and their survival was about the same. Only one of them had a presumable spontaneous remission. The only way we can tell we are making some progress is to use the group treated with steroids and antifolics as controls. Most of our cases admitted up until June of 1952 had the antibiotics that are available today, and they certainly had as many transfusions. There has been a considerable increase in the 50 per cent survival time with antifolics and steroids at about eight months and with mercaptopurine

and possibly azaserine additions which bring it to about twelve months, although antibiotics and transfusions and zeal may prolong life somewhat.

DR KIRSCHBAUM: To what extent is the difference between 6-mercaptopurine on the one hand and steroids and antifolics on the other due to selection of patients? Is it true that the acute case is treated with cortisone rather than with 6-mercaptopurine?

DR. BURCHENAL: In this series, this is just a question of time. Before 1952, June or January, depending on which series is considered, mercaptopurine was not available for treatment. Both of these series are consecutive cases, no matter how ill the patients were. In the second group, mercaptopurine was available if indicated. If the patient was very ill, he was still included in the series and treated.

DR. UPTON: I would like to make a comment which may or may not be out of place. Speaking of the therapy of leukemia, we are very interested at the Oak Ridge National Laboratory in the reversal or prevention of marrow injury by administration of nonirradiated bone marrow cells postirradiation. There is evidence that an irradiated animal will tolerate heterografts or homografts of marrow. Dr. C. C Congdon, who worked with Dr. E. Lorenz at the National Institutes of Health, tells me that he thinks they may have successfully treated some guinea pigs with lymphoid leukemia by whole body radiation Although one may not be ready yet to try this approach on a clinical level, I would be interested to know what you consider the potential outlook for this type of treatment in man

DR BURCHENAL: I think that sort of thing is very fascinating. I have not talked to Dr Congdon, but I did talk to Dr Lorenz about this Studies that we did at one time in attempting to sterilize mouse leukemia with radiation showed that total body radiation in a single dose of 2,500 r or above prevented transplantation of the strain of leukemia used On the other hand, at 2,000 r, some would transplant and some would not. At 1,500 r all would transplant Therefore, I question whether one can protect an animal against quite large enough doses to sterilize all leukemic cells It would be fascinating if you could, but it is my impression that Dr Lorenz was not able to do it, although survival time was prolonged

DR SCHLOSSER: Dr Collins has treated some patients in Houston with doses as high as 250 r in air in a single treatment This is about ten times the amount we usually give in a day I would like to ask Dr. Till how many patients they have now treated with busulfan, and how many of them have been treated a year or more Also, are you impressed that the survivals are as good as total-body or any other form of irradiation?

DR. TILL: We have only thirty-two patients in the whole series, including those in the early trials which started over five years ago. Four of these died

within the first year of treatment, two of these being in the early trials when excessive doses were used, and another died of pulmonary embolus. Our impression of survival of busulfan-treated cases so far is that this may be as good as for the other forms of treatment. We cannot say more with so small a series.

DR. KREMENTZ: Have there been any verified cases of leukemias which have spontaneously regressed?

DR BURCHENAL: They will certainly appear occasionally and can be very complete We saw one child recently who had a 100,000 white count which was alleged to be entirely monocytes The marrow was reviewed by an eminent pathologist, and then lost, unfortunately. At any rate, there seemed to be no question that it was leukemia at that time. Transfusions were given as a sort of a last resort, and the patient recovered and was perfectly well for three and a half years. Then she again presented a picture of leukemia and was treated with Amethopterin for about a month before we saw her. At the time we saw her, she was in remission and we stopped the drug. She lived another four years and finally developed fulminating leukemia, with no question concerning the diagnosis She died despite all available therapy Post-mortem examination confirmed the diagnosis. I am certain the first remission was spontaneous, the second one I suspect was spontaneous. We saw another girl who lived three and a half years after therapy with 2,6-diaminopurine, which is naturally a very effective agent. This may have been a spontaneous remission as well So they do occur, but they are rare, and I do not know any that have not recurred Of course, if a patient with a diagnosis recovers, the usual opinion is that it was only a leukemoid reaction originally. Spontaneous remission before steroids and antibiotics became available may have been due to infection with an accompanying stress reaction which may have resulted in a response in those very sensitive to steroid stimulus

DR. KIRSCHBAUM: Do you think that was a cortisone effect?

DR. BURCHENAL: I do not know, but would guess it probably was

DR SCHLOSSER How frequently should blood counts be done in patients receiving busulfan? Dr. Burchenal mentioned regression of large masses following 6-mercaptopurine, the development of high uric acid levels, and renal damage I would also like to hear some discussion of the problem of gouty kidneys.

DR BURCHENAL: Apparently, the fall in white count, regression of masses, and the increase in uric acid excretion depend on the agent used If an agent such as Amethopterin is used to treat chronic granulocytic leukemia, that agent is acting previous to the formation of the purine ring. For that reason, it seems to prevent the formation of any sort of purines which would then lead to uric acid, and the uric acid excretion level and level in blood decrease when therapy with Amethopterin is begun On the other hand, mercaptopurine, which interferes at a different level, prevents the conversion of hypoxanthine to the guanine of nucleic acid, and this is probably an accumulation of the hypoxanthine which

may be converted to uric acid and excreted as such. If x-ray or nitrogen mustard or possibly even the steroids are used, they seem to break down the nucleic acids at higher levels, and again an outpouring of uric acid may occur. As to therapy, I have no suggestions other than caution about giving too much of the agent and administering as much fluid as possible, particularly beforehand.

DR. ARROWSMITH: Nephrostomy has been reported as a lifesaving measure in these patients. I have seen two patients who apparently were anuric. One was a case of myeloid metaplasia thought to be leukemic and had one dose of x-irradiation over a massive spleen. His uric acid level was extremely high, and he was anuric for forty-eight hours but recovered spontaneously. The other one, a patient I saw with Dr Gould Andrews, had a massive lymphoma which quickly regressed with triethylene melamine and was accompanied by extremely high uric acid excretion and renal colic. This, as well as the previous case, subsided spontaneously after forcing fluids and without surgery, so I question whether nephrostomy is necessary.

DR. BURCHENAL: Most of the time it is not necessary.

DR. TILL: In answer to the question about the frequency of blood counts in patients taking busulfan, a count once in four weeks is probably sufficient, but for someone inexperienced in the use of the drug it is probably safer to have counts every two weeks.

DR. SCHLOSSER That would certainly be of no greater frequency than if x-ray therapy were used On the subject of high uric acid levels, we have observed patients in the past who were receiving irradiation over the spleen to collapse and expire within a few hours. Because of this, we now routinely start the dosage over the spleen extremely cautiously

DR KIRSCHBAUM Dr Schlosser, how do you explain the induction of remission in chronic leukemia by splenic irradiation?

DR SCHLOSSER: Of course, I do not really know However, some evidence now suggests that it is probably a chemical mechanism Whether it is necessary to irradiate the spleen or whether one can irradiate any area in the body and achieve the same results is another pertinent question It is also thought if blood supply is richer in a given area, that the responses may be better It is known, of course, in a wide variety of biologic materials that increasing the oxygen content of the medium increases the radiosensitivity of the organ I have been interested in this and have treated some recurrent breast tumors by having the patients breathe pure oxygen while half was irradiated, and irradiating the other half with no oxygen In two instances, I thought the oxygenated half regressed faster This is something that is very difficult to be positive about, however Since then, I have treated more cases in this manner and did not note any difference

DR. KIRSCHBAUM I am aware of the work on increased oxygenation, but I do not think you can explain any effects of splenic irradiation on the basis of

increased oxygen tension in the plasma. We are assuming that there is a significant effect. Do you think it is likely that, as a result of irradiation of the leukemic spleen, there may be something produced in the spleen which then is responsible for this systemic effect? I think it might be very worth while to see whether something might be extracted, let us say, from leukemic spleens of mice that have been irradiated

DR SYVERTON: Further comment may be helpful as it applies to the effects of irradiation X-irradiation of human epithelial cells in culture, normal or malignant, by use of between 700 r and 750 r, results in no visible cellular changes. The cells continue to metabolize and to produce virus. X-irradiation with from 1,000 r to 5,000 r stops multiplication, but cells continue to metabolize, and about half of them become hydropic and increase enormously in size. These cells, cell for cell, produce as much virus as nonirradiated cells. Internal cellular metabolic activity continues. Finally, from 15,000 to 30,000 roentgen units result in cellular shattering and total destruction.

DR. SCHLOSSER. Responses can be obtained no matter what portion of the body one irradiates. It is not necessary to irradiate the spleen

DR. KIRSCHBAUM: Why do you choose the spleen?

DR SCHLOSSER. Conventionally, one is dealing with an enlarged organ, and I have found the response to be better and faster if irradiation is directed toward the spleen. If there is not an enlarged spleen and there are enlarged mediastinal nodes, we start there

DR KIRSCHBAUM Why do you not irradiate normal tissues?

DR. SCHLOSSER I think it would probably work, although I have never tried it.

DR KREMENTZ Did Dr Kaplan do some work along this line with mice?

DR. KIRSCHBAUM: Kaplan's work was on the induction of leukemia. He irradiated thymectomized mice and postirradiation he transplanted thymus; in that thymus, leukemic transformation of the cells was introduced That was the secondary effect of radiation, perhaps peroxide formation or whatever the chemical mechanism is

DR KREMENTZ Dr Burchenal mentioned previously that the transplantation of human leukemic cells and lymph nodes to animals has not been achieved Would you enlarge upon this topic, Dr Burchenal?

DR BURCHENAL: We have transplanted sixty marrows from children with acute leukemia and from some adults as well, some of whom were certainly in the cortisone-resistant stage of their disease, into cortisonized hamsters, and none of them have grown The only thing we have been able to grow is a culture of leukemic cells which was originally isolated by Osgood from a case of monocytic leukemia These cells were grown in tissue culture and injected as a suspension into the cheek pouch A slight growth was noted in two weeks. By the end of twenty-eight days, there was a tumor approximately 5 mm in diameter growing

*in each cheek pouch. Sections of that tumor were diagnosed* as either anaplastic epidermoid carcinoma or reticulum cell sarcoma There is a question in everyone's mind as to whether the cells are leukemic or not. Dr. Osgood feels that the picture is perfectly consistent with a reticulum cell tumor, representing a solid form of monocytic leukemia. Others feel it is an epidermoid carcinoma. We would like to see transplantation of leukemic cells from human marrow into animals successful in 100 per cent of the cases. Then it might be useful as a screening agent Until that time, I question how much value it will be.

DR. KREMENTZ: Leukemic marrow has never been successfully transplanted into the anterior chambers of guinea pigs' eyes in our laboratory. I do not recall that anyone has ever successfully grown lymphomas on heterologous transplantation, either.

DR. UPTON: In relative ignorance of biochemistry, I would like to ask a question of the panel myself. Dr. Gellhorn presented some data showing changes in glucose tolerance associated with leukemia; there was a reversal of the abnormal glucose tolerance picture with remission. This calls to mind a recent paper by Warburg[10] concerning the altered respiration of tumor cells. Do other members of the panel have any evidence about this change in glucose metabolism in various types of leukemia? Can anyone say more about it at this stage? Is there a disturbance that is peculiar to patients with cancer and with diabetes, or does it occur in seriously debilitated patients under severe stress from other causes?

DR. BURCHENAL: The only evidence I know on it was that Dr Jorgen Kieler, from the University of Copenhagen, reported that he had studied human marrow by the Cartesian-diver technique, and found that the leukemic cells did respire. As to abnormal glucose tolerance, Dr. Glicksman's group at Memorial Hospital has seen that same sort of thing in patients with other types of cancer Incidence of abnormal glucose tolerance tests at Memorial Hospital in patients with cancer is higher than one would expect in the general population, so that is supportive evidence for Dr Gellhorn's work; but I think the fact in Dr Gellhorn's finding that when the leukemic cells were 100,000 to 400,000 in number there was an abnormal tolerance, and when they returned to normal numbers it also became normal, is much more exciting

DR. UPTON. Is this a disturbance that one sees which is peculiar to patients with cancer, or is this something occurring in seriously debilitated patients under stress from other causes?

DR. BURCHENAL. If I remember his criteria, they selected patients without diabetes and without a family history of diabetes who were not debilitated and were in good condition. This would be particularly important if additional proof cannot be obtained that an abnormal glucose tolerance is present before operation and a normal one is present afterward

*Walter J Burdette, PhD, MD*

# ETIOLOGY *and* THERAPY *of* LEUKEMIA*



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## EARLY HISTORY OF LEUKEMIA

| | | |
|---|---|---|
| 1845 | Disease first described. Named "leukemia" (λευκοσ + αιμα) by Virchow after the "farblosen Körperchen" | Virchow[98-100] Craigie[101] Bennett[101] |
| 1857 | Acute leukemia described | Friedreich[102] |
| 1858 | Leukemia first described in horse and pig | Leisering[103] |
| 1865 | Case of leukemia without characteristic blood picture reported | Cohnheim[104] |
| 1865 | First chemotherapy for leukemia (arsenic) | |
| 1866 | First splenectomy for leukemia | Bryant[105] |
| 1868 | Leukemia first described in birds | Roloff[106] |
| 1870 | Myelogenous leukemia with changes in the marrow recognized | Neumann[107] |
| 1871 | Leukemia first described in dog and cat | Siedamgrotzky[108] |
| 1872 | First attempt at transmission of leukemia experimentally, blood from human leukemia failed to induce leukemia in dogs and rabbits | Mosler[109] |
| 1874 | Leukemia first described in mouse | Eberth[110] |
| 1879 | Rational classification of various forms of the disease outlined as a result of microscopic studies of stained preparations, as well as macroscopic observations | Ehrlich[111] |
| 1900 | Myeloblast identified, providing means for distinguishing between acute and chronic myelogenous disease | Naegeli[112] |
| 1903 | First successful treatment of leukemia with roentgen therapy | Senn[113] |
| 1908 | Avian leukosis transmitted by cell-free agent | Ellermann and Bang[27, 114] |

**ETIOLOGY**

A relative and absolute increase in acute leukemia[115] and in leukemia in the group of patients more than 50 years of age[116] has been reported. This increasing incidence of leukemia in recent years is greater only for one other malignant disease, cancer of the lung. In the United States, the mortality rate from leukemia, which was 10 per million in 1900, rose to 63 per million in 1953. The disease is relatively common in the first decade of life, and it is then encountered less frequently until the fifth and sixth decades are reached. Lymphocytic leukemia predominates in childhood and in the older age groups, but myelocytic leukemia exceeds lymphocytic leukemia between the ages of 20 and 50 years. In most series of cases there is a preponderance of males,[117, 118] and among racial differences[119] the incidence among Negroids is considerably less than among Caucasoids.[117, 120] The death rate for the two groups is equal in the nonwhite population, but lymphocytic leukemia is responsible for 41 per cent of the total deaths and myelocytic leukemia for 33 per cent of the total deaths from leukemia among Caucasoids.[113] Although the familial occurrence of leukemia is sporadic and has not been reported as frequently as for many other neoplastic diseases, a number of reports support the premise that genetic factors for susceptibility are operative in the human disease.[9, 121 124] Leukemia has been observed in siblings,[125] in twins,[126] and two varieties have appeared in a single family.[127] The age of onset often is similar for different, affected members of a family group. Insufficient data are available for any unequivocal view on the number, location, or kinds of genes involved,[123, 128] however.

Leukemia, more commonly chronic than acute, occurs infrequently in pregnancy.[129-135] The prognosis is better if pregnancy occurs early in the course of the disease. Apparently, the child neither contracts the disease from the mother, nor has the mother been affected in the rare cases of congenital leukemia.[136-139]

A century after the original observations of Craigie, Bennett,[101] and Virchow,[98-100] who recognized the disease independently, the first encouragement that the disease can be controlled since the advent of radiotherapy[113] is forthcoming. Diagnostic methods have progressed more slowly since observations by these pioneers and the microscopic observation of Donné,[140] Ehrlich,[111] Friedreich,[102] and Naegeli.[112] It is only within the last decade that the observations of Ellermann and Bang[27, 114] a half century ago have been extended to any degree in mammals, and evidence that viruses are associated with the origin of human leukemia remains equivocal. However, steady progress has been made in expanding knowledge about the role of filterable agents in inducing other tumors in animals and plants.[12, 14, 15, 18, 19, 21, 43, 72, 141, 142] No longer are articles encountered attributing the onset of leukemia to trauma.[143-146]

Possibly the experiments which have stimulated the most work and speculation in recent years have been those of Gross.[1, 24, 28, 39, 40-42, 44, 46, 48, 49, 141, 147, 149] He was able to prepare extracts from leukemic tissues in the AK strain of mice by centrifuging and passing the material through Selas filters, which then was effective in producing leukemia when inoculated into newborn animals. This has been confirmed by Stewart,[26] Woolley and Small,[43, 50] Woolley,[52] and others. Not only were extracts prepared in this manner effective in producing leukemias, but also parotid tumors and sarcomas of soft tissues appeared and apparently depended on the introduction of a virus or viruses. The induced tumors which appeared were of the type similar to those appearing in the recipient rather than in the strain of mouse from which the agent was derived. Stewart and co-workers[149] were able to culture this agent on renal tissue from monkeys in vitro. This agent obtained from mouse parotid tumors and leukemias was enhanced in effectiveness by serial passage. Friend[51, 151, 334] noted the cytoplasm of Ehrlich ascites tumor cells which were carried serially in Swiss mice contained particles suggesting inclusions which are commonly seen in cells infected with viruses. Following this lead, she was able to extract a cell-free agent which can be transmitted in adult mice. The virus produces a disease having the characteristics of leukemia, although the origin of the mononuclear cells seen characteristically in the peripheral blood of affected animals has not been determined. Schwartz[142] and Schwartz and associates[152] reported the isolation of a filterable agent from the brain of patients dying with leukemia which will accelerate the development of leukemia in AKR mice and induce leukemia in white Swiss mice Beard[154] clarified the etiology of avian leukosis, finding distinct but related viruses associated with avian leukemia, myeloblastosis, and erythroblastosis The three agents of leukosis in turn are but part of a larger system of agents responsible for the numerous avian lymphomas.

Interest in the etiology of human leukemia has focused on the relation of irradiation to the origin of the disease It is eight to ten times as common in radiologists as in other physicians[335-337, 340] The incidence of the disease has increased alarmingly in the last twenty years concomitant with the use of x-irradiation, but the increment became apparent before the advent and common usage of radioactive isotopes. Evidence obtained from victims of the bombing of Hiroshima indicates that a single large dose of irradiation may result in leukemia Moloney and Kastenbaum[164] reported that leukemia is twelve times as common in survivors near the hypocenter at Hiroshima and Nagasaki as in survivors at the periphery. Additional evidence that leukemia may occur as a result of x-ray treatment and diagnostic procedures is found in the studies[illegible] on individuals with leukemia treated for spondylitis by x-ray in Holland and Great Britain. Also leukemia has been reported following irradiation of the mediastinum in childhood.[160] Difficulties in assessing and governing the use of therapeutic and diagnostic procedures with radiation are the defi-

ciencies in our knowledge concerning maximum, safe dosage over various intervals of time and the biologic effects of extremely low dosage of irradiation. Current inquiry into possible deleterious effects of the amounts of irradiation commonly acquired by the population at large is exceedingly disturbing.[339-341]

The evidence from animal experimentation supports the inference that leukemia may occur in human beings as a result of irradiation. Furth and Furth[31] were the first to demonstrate the induction of leukemia by x-irradiation in mice with a low incidence of leukemia. The experiments which Upton and Furth[34] reported in the RF strain are particularly convincing, since the type of leukemia appearing after irradiation is myeloid, whereas the type of spontaneous leukemia encountered in the strain is lymphoid. The induced type occurs with lower dosages most frequently in males, whereas the spontaneous leukemia occurs predominantly in females. Although lymphatic leukemia may originate extrathymically in old age,[194] spontaneous and induced lymphomas usually originate in the thymus, since early thymectomy reduces the incidence in most strains of mice.[59, 342-344] In the RF strain, however, thymectomy did not alter the incidence of myeloid leukemia induced by irradiation, suggesting that the thymus is the source of lymphogenous leukemia only. Partial shielding[61, 62] of bone marrow[61] and spleen,[62] administration of untreated, isologous, marrow cells,[63] and fractionation of dosage inhibit the induction of lymphoid leukemia by irradiation. Estrogen may offset the protective effect of shielding bone marrow but does not exert a similar effect on thymic shielding in C57BL mice.[345] Partial shielding[67] and dividing the dosage of irradiation also inhibit the myeloid leukemogenic effects of x-ray treatment. Kaplan and coworkers[68, 346] demonstrated that leukemia will appear in thymectomized and irradiated mice, which ordinarily do not develop leukemia, when they are inoculated subsequently with thymic tissue which has not been irradiated. Therefore, the effect is apparently not a direct one on the thymic cell. Genetic *susceptibility to pulmonary tumors*[137, 139] *resides within the target tissue when* it is transplanted, rather than being a systemic influence. Similarly, thymic cells retain their property for promotion of leukemia even though the leukemic cells themselves may arise from recipient tissue. For example, Law and Potter[69] found that leukemia appeared in thymectomized (C57BL × A) $F_1$ hybrids when thymus was transplanted from the susceptible (C57BL) parent and not when grafts from the resistant (A) parent were used. Progenitor cells, as determined by histocompatibility characteristics, originated most frequently from the $F_1$ host and less frequently from the nonirradiated C57BL graft.

Snijders[29] and Tio Tjwan Gie[35] performed the first experimental studies on leukemia in mammals. They transmitted the disease in closely related guinea pigs, producing lymphosarcoma after local inoculation and leukemia after intravenous passage. Transplantation between strains is possible early in development before the mechanism of immunity[28, 345] is mature, and both

irradiation[349, 350] and cortisone, acting alone or synergistically,[351] may reduce the immunologic response to transplantation of leukemic cells into foreign strains and species. Natural resistance to mouse leukemia may be transferred from rats without treatment to irradiated rat parabionts[352] which otherwise would have been susceptible, but not from a resistant mouse to a susceptible parabiont without treatment.[353] Serial passage of leukemia in mice[354] can result in alteration of cellular virulence without concomitant changes in morphology, and the serum of immune animals can inactivate leukemia cells in vitro.[355] Previous ideas about specificity and immunity have been challenged sharply by the polyomas encountered when Stewart and associates[356] inoculated hamsters with extracts from mouse leukemia.

Pronounced differences in susceptibility to leukemia between inbred strains of mice were noted during the early work of Richter and MacDowell[30, 156] with the C58 strain, although nongenetic factors were also known to influence the appearance of leukemia since all of the genetically susceptible C58 mice did not develop the disease They were also able to demonstrate a maternal factor producing resistance to the development of leukemia so that incidence was reduced and latent period was prolonged. Although C58 mice have a high incidence of spontaneous leukemia, they are refractory to treatment with methylcholanthrene[356]; whereas DBA/2 mice are susceptible to the leukemogenic action of methylcholanthrene but have a low incidence of spontaneous leukemia[357] Usually, $F_1$ hybrids show susceptibility to spontaneous and induced leukemia intermediate to that of susceptible and resistant parental strains, the degree depending on the specific strains used, and multiple genes for susceptibility are operative.[358, 359] When two strains susceptible to estrogen leukemogenesis are crossed, the susceptibility of $F_1$ mice may be greater than that of either parent, however.[16] Also, the $F_1$ animals may be resistant to the effect of estrogen when susceptible and resistant parents are mated.[338] Age[360] alters susceptibility to induction of leukemia by transplantation of leukemic cells,[29, 348] transmission by virus,[39, 361] and induction by irradiation[362] and carcinogens[363] As a rule, leukemogens are most effective when administered at an early age, and resistance in $F_1$ hybrids is intensified with increasing maternal age[364, 16]

Numerous studies have substantiated the increased incidence of leukemia in susceptible strains following administration of carcinogenic hydrocarbons[3, 347, 365] and estrogen[illegible] Extrathymic lymphoid tumors appear after thymectomy in DBA 2 mice treated with methylcholanthrene,[60, 342] and Kirschbaum reported that lymphosarcoma from the C3H strain can be grafted into DBA mice provided they are painted with methylcholanthrene[16] Urethan will increase the effectiveness of other leukemogens, although it is not leukemogenic alone.[366] Estrogen augments the leukemogenic action of irradiation,[367] but androgen may counteract this action of estrogen as well as diminish that of methylcholanthrene[368] and irradiation.[369] On the other hand, castration enhances susceptibility to spontaneous leukemia[44, 370] and leukemogenic effects

of methylcholanthrene.[269] Contrary to the preponderance of leukemia in human males, it is interesting that thymomas and lymphocytic leukemia occur most frequently in female mice.[344, 370] Adrenalectomy increases the susceptibility of mice and rats to spontaneous and transplanted leukemia[371, 372] and to that induced by irradiation,[373] but cortisone delays the onset of spontaneous lymphocytic[54, 374] leukemia, thymic lymphosarcoma induced by x-irradiation,[54, 375] and leukemia induced by methylcholanthrene[376] if dosage is sufficiently great and prolonged. Administration of pituitary growth hormone is associated with the appearance of pulmonary lymphoid tumors in rats if the hypophysis is intact,[376] but tumors do not appear in hypophysectomized animals.[377] The enhancement of suceptibility to leukemia in mice by thyroid deficiency may be mediated by effects on body weight, since there is a direct correlation between weight and the appearance of leukemia.[378] Reduction of caloric intake alone diminishes the number of spontaneous lymphomas and prolongs the mean time of survival in transplanted leukemias.[159, 379]

Interest in leukemia in experimental animals is heightened by the similarity between the disease in the mouse and in man In summary, experimental studies suggest that susceptibility to leukemia in animals is due to multiple genes governing not only the spontaneous incidence but also response to various agents. Incidence is increased by irradiation, the presence of virus, estrogen, carcinogenic hydrocarbons, castration, adrenalectomy, and perhaps pituitary growth hormone under certain conditions Combination of different agents may act synergistically, and urethan, although inactive alone, potentiates the action of leukemogens Leukemia is inhibited by increasing age, caloric restriction, cortisone, androgen, thymectomy, and shielding of bone marrow, thymus, and spleen following irradiation Growth of leukemic cells in foreign strains may be promoted by inoculation early in development, cortisone, irradiation, and carcinogenic hydrocarbon Both the leukemogenic effects of irradiation and the intrinsic property of tissue from genetically susceptible donors to initiate the disease are apparently exerted indirectly

## DIAGNOSIS AND CLASSIFICATION

Classification of the leukemias is often controversial if detailed and too superficial if simple Most of the difficulties in regimentation stem from uncertainties due to the limitations of morphologic criteria Sprague has emphasized the possibilities for differentiating between myeloid and mononuclear series by means of ribonuclease and desoxyribonuclease In the future, pertinent information extending knowledge about the varieties of human leukemia should be forthcoming by means of cytochemical preparations, tissue culture, and microcinematography, utilizing electron, phase, reflecting, and interference microscopy.

The nomenclature used in this chapter (Table XXIII) includes myelogenous, lymphogenous, monocytic, and stem cell forms of leukemia, designated ac-

TABLE XXIII

NOMENCLATURE OF LEUKEMIAS

| TYPE | CLINICAL COURSE IN RELATION TO PREDOMINANT CELL: ACUTE | CHRONIC |
|---|---|---|
| Myelogenous | Myeloblast | Myelocyte<br>Neutrophil<br>Eosinophil<br>Basophil |
| Lymphogenous | Lymphoblast | Lymphocyte |
| Monocytic | Monoblast | Monocyte |
| Stem cell | Undifferentiated stem cell | |

cording to the predominant type of cell and based on studies both of bone marrow and peripheral blood. In addition, the disease is divided temporally into the acute form, with blasts predominating, and into the chronic form of the disease, with either lymphocytes or myelocytes chiefly. Plasma cell leukemia has not been included because of uncertainties concerning its identity with multiple myeloma[160] The leukemic manifestations of malignant lymphoma have also been omitted. In unusual cases of the chronic myelogenous disease, the eosinophil or basophil may be the most common type of cell. However, the manifestations of leukemia range from a rather pure form to erythroleuko-piastrenemia, and from cases with pronounced leukocytosis to subleukemic forms of the malady.

## SCREENING FOR CHEMOTHERAPEUTIC AGENTS

Possibly because the leukemias occurring in laboratory animals fairly closely correspond to those appearing in the human population, remarkable success has been achieved in a relatively short time in the treatment of leukemia Despite problems in evaluation due to advances in supportive therapy, variations in sequence and time of treatments, and scarcity of valid data on untreated cases, it is clear that the survival time of patients with leukemia has been prolonged by chemotherapeutic agents[161] The hope that the difference in response between leukemic and normal cells will lead to cure of the disease, therefore, has some justification

In general, initial screening has consisted of testing an exceedingly sensitive transplantable tumor such as sarcoma 180 or the Walker carcinoma 256 for susceptibility to the compound chosen However, no single tumor can be used as an effective screen, and a spectrum of spontaneous and transplantable tumors should be used for an adequate survey, as well as biologic systems for testing certain synthetic steps or cytogenetic effects Suitable, active material then is used for clinical investigation after it has been tested for possible toxicity So far, screening procedures have failed to yield a good correlation between activity of compounds in the tumor systems and in the nontumor systems of testing, indicating that the antitumor effects detected are somewhat specific.

However, clinical effectiveness has been encouraging when promising compounds have been tested, and occasionally compounds are more effective against human, neoplastic disease than experimental tumors in animals. Past deficiencies in the method may be attributed as much to the paucity of agents completely effective against experimental tumors such as leukemia as to an inadequate approach to screening.

## TREATMENT

The quest for efficient therapeutic agents has not only been empiric, but rational selection of compounds to be tested more frequently has ultimately led to successful treatment. Choice of compound has been combined with a fortunate range of methods for testing effectiveness in biologic systems. The therapeutic effects of alkylating agents and antimetabolites which were chosen for testing in this fashion[162-164] indicate the importance of knowledge concerning pathogenesis of leukemia in selecting the proper system for screening, as well as the selection of agent to be tested.

### IRRADIATION

The oldest[113] consistently successful treatment for leukemia is irradiation. Careful comparisons by a number of therapists in the past[115, 165-179] have led to the conclusion that irradiation prolongs the useful and efficient portion of the patient's remaining life span, but the average total duration of life probably is unchanged in irradiated groups. In general, irradiation has been confined to chronic forms of the disease, but Schlosser suggests irradiation may now be combined with chemotherapeutic agents even in acute leukemia. For example, steroid therapy may be used to curb a crisis and irradiation tried subsequently to prolong the remission. This plan of combined treatment for acute leukemia is not universally acceptable to all therapists at the present time, however. The usual form of roentgen therapy for leukemia is irradiation of the spleen, irradiation of lymph nodes or visceral areas, irradiation of large segments of the body, and total-body or body-bath irradiation. $P^{32}$ therapy [97, 166, 180-188] has the advantage of not being associated with radiation sickness, and it requires few special facilities. Roentgen therapy is superior, however, *when reduction in size of spleen and lymph glands is desirable*[187]. $P^{32}$ may be administered either orally or intravenously, and the usual dosage is 0.5 to 2 mc, with intervals between successive doses selected according to response. Therapy with radium,[189] radioactive gold,[190, 191] and radioactive sodium[192] has been reported, but it is not widely employed for leukemia.

The method of titrated, regularly spaced roentgen or $P^{32}$ therapy described by Osgood[193] has resulted in survival which is superior to other programs of therapy, according to the statistical analysis of Tivey. The salient features of this plan are therapy immediately after the diagnosis is made, irradiation

TABLE XXIII
NOMENCLATURE OF LEUKEMIAS

| TYPE | CLINICAL COURSE IN RELATION TO PREDOMINANT CELL | |
|---|---|---|
| | ACUTE | CHRONIC |
| Myelogenous | Myeloblast | Myelocyte<br>Neutrophil<br>Eosinophil<br>Basophil |
| Lymphogenous | Lymphoblast | Lymphocyte |
| Monocytic | Monoblast | Monocyte |
| Stem cell | Undifferentiated stem cell | |

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of the entire body either by roentgen therapy or $P^{32}$, titration of the dose depending on the response of the patient, and initial weekly interval between dosage altered until suitable maintenance dosage and interval are obtained.

When leukemia is being managed with chemotherapy, roentgen irradiation is useful supplemental therapy for refractory, localized disease. Localized lesions in the bones which are painful, enlarged spleen, localized lymphatic involvement, enlarging kidneys, and meningeal infiltration, giving signs of increased intracranial pressure, all respond to irradiation with sufficient frequency to justify trial Variations in the sequence, treatment factors, dosage, and field irradiated are numerous and depend on the inclinations and experience of the radiologist, as well as the response of the individual treated. For example, doses of total-body irradiation, although usually in the range of 10 to 20 r, sometimes are given in massive amounts;[194] and irradiation directed to the spleen usually is 200 to 400 r, but it may be as great as 2,000 r in refractory cases. More irradiation may be required for a response in myelocytic than in lymphocytic leukemia. Edema may follow irradiation of enlarged lymph glands, and this can prove disastrous when impending venous or tracheal obstruction is present.

The possibilities for improvement of therapy inherent in investigation of the effects of irradiation on normal and neoplastic tissues are as great as continued chemotherapeutic studies Actually, some of the effects of irradiation and chemotherapeutic agents are strikingly similar. Regression of tumors at a distance from the field of irradiation and changes in nonirradiated tissue transplanted to an irradiated field suggest humoral mediating factors. The reversibility of irradiation damage by transplantation of bone marrow and the protective effect of shielded tissues have been studied extensively.[61, 87, 195] The proposal that damaged cells are repaired by a process akin to transduction and transformation[196] has not been confirmed, but methods of labeling cells[64] and information about mutagenic peroxides formed in irradiated material, as well as other studies on altering radiation effects, show real promise.[74, 197]

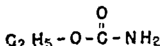
CHEMOTHERAPY

*Benzene.*—Exposure to benzene (benzol) may result in changes in the entire hematopoietic system, the most characteristic of which are found in the bone marrow Hyperplastic reactions occur more commonly in males and hypoplastic changes in females Chronic exposure is associated not only with anaplasia but also, less commonly, with leukemia.[198] In the past, experience showed it to be useful treatment for leukemia[199] in daily dosage of 2 to 5 Gm Now it is no longer used therapeutically because of potential toxicity and the advent of more satisfactory compounds

*Arsenic*—One of the toxic effects of arsenic is to depress the leukocyte count, and compounds releasing inorganic arsenic have been employed in the

past for the treatment of leukemia.[199, 200] Best results were obtained in chronic myelocytic disease during continuous therapy. Fowler's solution, N.F. (potassium arsenite), usually was given in doses increasing from 0.3 mg. three times daily until response or toxic effects ensued In addition to oral and rectal routes of administration, potassium arsenite also was administered intravenously in dilute solution. This treatment has been superseded largely by other forms of therapy.

*Urethan.*—Ethyl carbamate, which has been known for many years as a hypnotic, was tested for possible inhibition of various types of tumors by Haddow and Sexton[201] as part of a general program of screening of mitotic inhibitors. Leukopenia was observed, and Paterson and associates[202] subsequently reported response of leukemia to administration of the drug. The exact mode of action has not been clarified by tracer studies with labeled urethan, but it is of interest that the compound is carcinogenic in animals.[203] Urethan is not effective in acute leukemia, and chronic myelocytic leukemia responds to it



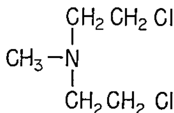
Urethan
(Ethyl Carbamate)

more favorably than chronic lymphocytic leukemia There is a lag in leukocytic response, so that the discontinuation of therapy is prudent when the count reaches fifteen to twenty thousand leukocytes per cubic millimeter. The usual dose of the drug is 1 Gm after meals three times daily, but there is considerable variation in response from patient to patient Smaller dosage may be effective in maintaining remissions In favorable cases, the total leukocyte count recedes to the normal range, the differential count approaches the normal pattern, the hemoglobin level rises, and the spleen and enlarged nodes are reduced in size Toxic effects[204] are most commonly nausea and vomiting, but may also include hypoplasia of the bone marrow, hepatic injury, and damage to the central nervous system

*Alkylating Agents.*—

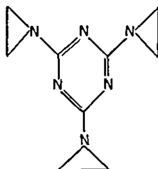
*Mechlorethamine Hydrochloride* Therapeutic possibilities of the radiomimetic nitrogen mustards were first recognized by Goodman and associates,[205] Goodman and Gilman,[206] and Gilman and Philips,[207] who found that transplanted lymphosarcoma regressed after treatment of mice with crystalline hydrochlorides of the nitrogen mustards Later, Lindskog[208] obtained clinical remission in a patient with lymphosarcoma, and hematologic studies by Dougherty showed reversible depression of bone marrow function Lymphocytopenia usually precedes granulocytopenia [208] Methyl-*bis* ($\beta$-chloro-

ethyl) amine hydrochloride ($HN_2$, Mechlorethamine hydrochloride, Mustargen) causes a response in both chronic lymphogenous and chronic myelogenous leukemia[208-213] which may be prolonged, but chronic lymphocytic leukemia accompanied by anemia seldom responds satisfactorily,[210] and acute leukemia cannot be managed with Mustargen. The usual course of therapy consists of 0.4 mg. in fresh solution per kilogram of body weight. Mustargen was used in far-advanced lymphogenous leukemia refractory to irradiation and in disseminated disease with visceral involvement.[213] Because both roentgen therapy and other chemotherapeutic agents yield longer remissions, Mustargen now is seldom used even in combination with other treatment.[211, 212, 214]

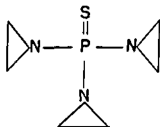



Mechlorethamine
($HN_2$, Mustargen, Methyl-bis
[β-Chloroethyl] Amine)

*Triethylene Melamine.* Triethylene melamine (2,4,6-triethylene-*s*-triazine; TEM)[215, 224] is a congener of Mustargen which is effective in chronic lymphogenous and myelogenous leukemia. Acute leukemia does not respond




Triethylene Melamine
(TEM, 2,4,6-Triethylene-*s*-Triazine)




N-N′-N″-Triethylene Thiophosphoramide
(ThioTEPA, TSPA)

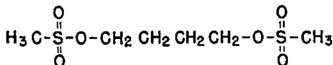
This compound was synthesized by textile chemists and tested independently in England and in the United States. Inhibition of neoplastic growth in animals was reported by Rose, Hendry, and Walpole,[225] Lewis and Crossley,[226] Burchenal and co-workers,[227] and Philips and Thiersch.[228] Clinical trials[229-231] were immediately encouraging. One of the chief advantages of the drug is the fact that it is a nitrogen mustard analogue which can be administered orally.[217] It

may also be given in one-half to one-third the oral dose intravenously. The compound, in acid solution, forms ethyleneimonium cations which are biologically active and cytotoxic. For that reason, it is administered orally approximately two hours before breakfast, and 2 Gm. sodium bicarbonate is given with each 5 mg. triethylene melamine in order to prevent their appearance in the stomach.[224] Injections of triethylene melamine into the pleural cavity can be effective in preventing the reaccumulation of pleural fluid.

Nausea and vomiting are less troublesome with triethylene melamine given by mouth[232] than with other nitrogen mustard therapy. Considerable delay in action of the drug occurs following administration, and successive doses must be given cautiously, especially if it is being given on an outpatient basis. Ulceration of the intestinal mucosa, thrombocytopenia, and hemorrhage[215] have been reported in patients receiving triethylene melamine Higher doses are required to obtain a comparable response in chronic myelocytic than in chronic lymphocytic leukemia. Dosages recommended vary somewhat, but an initial dose up to 5 mg. in chronic lymphocytic leukemia and up to 10 mg in chronic myelocytic leukemia is reasonably safe. Subsequent treatment can then be planned on the basis of response within the period of a week or more. Triethylene melamine is now rarely used for the treatment of chronic myelogenous leukemia

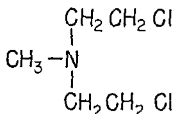
*N-N'-N'' Triethylene Thiophosphoramide*: Shay and associates,[233] Zarafonetis and co-workers,[234] and Leonard and associates[218] reported response of chronic lymphogenous and chronic myelogenous leukemia to administration of triethylene thiophosphoramide (thioTEPA). Acute leukemia not only does not respond, but also the experience of Smith and associates[235] led them to advise that thioTEPA is contraindicated for children with acute leukemia The drug is useful because it can be given intramuscularly and orally when the intravenous route is not desirable or practical Local tumefaction, hepatomegaly, splenomegaly, and leukocytosis regress with treatment Anorexia, nausea, and vomiting may occur, and therapy should not be given when thrombocytopenia is encountered A course consists of three to four weekly treatments of 25 mg and should not be repeated for one month to six weeks [218]

*Busulfan.* Busulfan (1,4-dimethanesulfonyloxybutane, Myleran, G T-41) is a polyfunctional alkylating agent depressing the granulocytic series which was synthesized by Haddow and Timmis in 1953[82] and found by Galton and



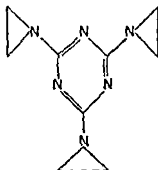

Busulfan
(GT-41, Myleran, 1,4-Dimethanesulfonyloxybutane)

ethyl) amine hydrochloride ($HN_2$, Mechlorethamine hydrochloride, Mustargen) causes a response in both chronic lymphogenous and chronic myelogenous leukemia[208-213] which may be prolonged, but chronic lymphocytic leukemia accompanied by anemia seldom responds satisfactorily,[210] and acute leukemia cannot be managed with Mustargen. The usual course of therapy consists of 0.4 mg. in fresh solution per kilogram of body weight. Mustargen was used in far-advanced lymphogenous leukemia refractory to irradiation and in disseminated disease with visceral involvement.[213] Because both roentgen therapy and other chemotherapeutic agents yield longer remissions, Mustargen now is seldom used even in combination with other treatment.[211, 212, 214]

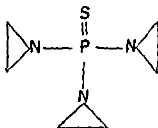



Mechlorethamine
($HN_2$, Mustargen, Methyl-*bis*
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Considerable variation in the amount necessary for a response has been encountered, and whether irregularity of absorption or utilization is responsible is not known. Dosage has varied from 0.1 to 13 mg. per kilogram of body weight. Damage to bone marrow is more likely during continuous therapy than after intermittent administration, but suppression of hematopoiesis usually is reversible.[260] Lower dosage is advisable when initial thrombocytopenia is found, and such instances constitute a group in which chlorambucil is particularly useful.[89, 259] *Anorexia, nausea, and vomiting occur only with large doses of the* drug. When properly administered, chlorambucil rarely causes undesirable side effects.[89] Approximately two-thirds of the cases of chronic lymphocytic leukemia treated respond favorably[259] with decrease in leukocyte count, correction of abnormalities in differential count and hemoglobin level, and diminution of hepatosplenomegaly and lymphadenopathy. Response occurs usually by the fourth week of therapy and may last as long as six months. Although a shorter course of triethylene melamine may be required for remission, triethylene melamine is associated with more untoward side effects than chlorambucil. The superiority of chlorambucil to irradiation is questionable,[254] and additional experience will be required to establish its most useful role in the therapy of leukemia.

*Antimetabolites.—*

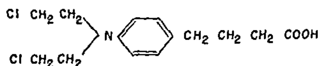
*Folic Acid Antagonists.* The deleterious effects of folic acid when administered to patients with leukemia[261, 262] are well known. After the structure and function of this vitamin were established, analogues became available, and one of the striking effects of these antimetabolites proved to be leukopenia.[263] The 4-amino analogues, 4-aminopteroylglutamic acid (Aminopterin), 4-amino-$N^{10}$-methylpteroylglutamic acid (Methotrexate, Amethopterin), and 4-aminopteroylaspartic acid, were found to be active in mammals. However, the administration of pteroylglutamic acid (PGA) does not reverse the effect of folic acid analogues in mammals because pteroylglutamic acid is transformed into folinic acid (citrovorum factor) in the process of nucleic acid metabolism. When the citrovorum factor is used to combat toxicity, it nullifies the therapeutic effects of folic acid antagonists as well.

The successful use of folic acid analogues in acute lymphoblastic and myeloblastic leukemias was first reported by Farber and associates.[261] *Some response is* obtained in approximately two-thirds of children treated,[264, 265] and best results are obtained in acute lymphoblastic disease. Chronic leukemias rarely[266] respond. Of the 4-amino analogues, Methotrexate is the most satisfactory.[265, 267] Toxic side effects of continued therapy are stomatitis, diarrhea, pancytopenia, portal cirrhosis, and alopecia. The alopecia is reversible during therapy. Folic acid antagonists should not be given during pregnancy because of deleterious effects on embryogenesis.[268] When renal insufficiency is present, plasma levels may con-

Till[93, 236] to be effective palliation for chronic myelogenous leukemia. The effectiveness of busulfan has been confirmed by numerous investigators,[84, 86, 237-253] and it generally is regarded as superior to triethylene melamine, urethan, and other chemotherapeutic agents. More experience will be required to determine how this drug compares to irradiation, but it seems to be equally effective Busulfan is without significant toxicity when given properly, but excessive single and loading doses are unwise since thrombocytopenia and hypoplasia of the bone marrow may ensue. Splenic irradiation may be necessary in addition to busulfan. Till cautions against continued dosage during pregnancy.

Although patients may not be alert to symptoms of the disease and experience relief after therapy, Till advises against beginning therapy in the absence of symptoms. The leukocytic response may be delayed as long as six to eight weeks, particularly if splenic irradiation has preceded therapy, but usually occurs before this time. Increase in hemoglobin level may not begin earlier than one month and may not reach normal levels until three months have passed since the beginning of therapy, depending on the initial level but independent of the leukocytic response. Responses may last as long as twelve months. Relapses occur earlier with successive courses of therapy, and eventually continuous administration of the drug may become necessary The recommended oral dosage is not greater than 0.065 mg per kilogram of body weight, which is approximately 4 mg daily for most patients. Dosage greater than 6 mg may depress myelopoiesis and offers no advantage. The biologic specificity of busulfan, although not absolute, and its occasional effectiveness in the management of cases refractory to radiotherapy make it exceptionally useful in the management of chronic myelogenous leukemia

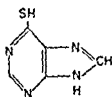
*Chlorambucil*· In the course of investigating biologic effects of aromatic alkylating agents on normal and neoplastic tissues, Haddow[164, 254] found that a paraphenylbutyric acid derivative of mechlorethamine, chlorambucil (CB-1348, p-NN-[di-2-chloroethyl] aminophenylbutyric acid), inhibited the Walker



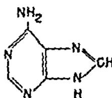

Chlorambucil
(CB-1348, p-NN-[di-2-Chloroethyl]
Aminophenylbutyric Acid)

carcinoma 256[255] Further studies by Elson[255] and also by Bollag[256] revealed that the agent depressed the lymphocyte count in the rat without a comparable change in the granulocytes Experience by Galton and associates[89] and others[257-259] soon demonstrated the usefulness of chlorambucil in chronic lymphocytic leukemia.

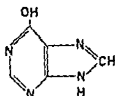
also respond occasionally. Busulfan and irradiation give superior results in the therapy of chronic myelocytic leukemia. Clinical trials followed the demonstration by Clarke and associates[96] that the drug not only inhibited but also cured sarcoma 180 and increased the survival of mice with transplanted leukemias, both sensitive and resistant to Amethopterin. Some children resistant to therapy with Amethopterin and steroid therapy responded to 6-mercaptopurine also. The dosage of 2.5 mg. per kilogram of body weight per day usually amounts to 5 mg. in a child and from 100 to 200 mg. in an adult. The drug must be given for long periods to be effective. Remissions are not expected in less than from three to eight weeks, and Burchenal advises treatment at least eight weeks before abandoning trial with 6-mercaptopurine. On occasion, complete remission may not occur for ten to twelve weeks. Although toxicity at the recommended dosage is minimal, bone marrow hypoplasia does occur, and nausea and vomiting are other undesirable side effects. Renal damage as a result of high uric acid levels following rapid fall in leukocyte count should be anticipated by regulating the amount of the drug administered. Remissions



6-Mercaptopurine (Purinethol)



Adenine



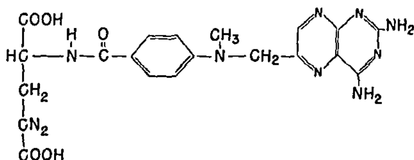
Hypoxanthine

Purine Analogues

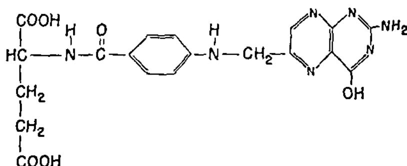
usually are shorter in childhood acute leukemia than remissions following therapy with folic acid antagonists. Combined therapy with the glutamine antagonist, azaserine,[286] gives results suggestive that complete remissions are somewhat longer than those with mercaptopurine alone.[161] Hill and Vincent[91] reported that large doses of fluorohydrocortisone in addition to 6-mercaptopurine may give remissions in adults with acute leukemia.

*Steroids.*—Cortisone, hydrocortisone, ACTH, prednisone, and prednisolone may produce prompt remissions and are particularly valuable in acute leukemia occurring in children. Farber and co-workers[287, 288] and Pearson and associates[289] were the first to recognize the usefulness of steroids in leukemia. In comparison to the other steroids, prednisone and prednisolone produce comparable therapeutic effects with less undesirable changes in electrolyte balance.[290] Bernard and Deltour,[291] Hill and associates,[91, 292] and others[92, 293, 294] administered cortisone in doses up to 5 Gm. and prednisone and prednisolone in doses up to 1 Gm. daily in adults to produce responses which would otherwise not be achieved. On the basis of response of the leukemia, there may be some justifica-

tinue to be high, and care should be exercised in calculating dosage to prevent cumulative toxic effects.[269] Methotrexate may be given intramuscularly or orally in daily doses of 1.25 to 5 mg. The drug can be continued in daily doses up to two months,[161] or until remission occurs. If continuous therapy then is elected, treatment on alternate days with lower dosage may be advisable. Serial studies of bone marrow and peripheral blood are advisable for most satisfactory management. Although relapse inevitably occurs and remission may be of only a few weeks' duration, the response may extend over a period of many months in favorable cases.[261, 264, 265, 270-273] In general, remissions are longer than after therapy with 6-mercaptopurine and steroids.[161]




Amethopterin
(Methotrexate, 4-Amino-N[10]
Methylpteroylglutamic Acid)




Folic Acid
(PGA, Pteroylglutamic Acid)

Folic Acid Analogues

*Purine Antagonists* Utilizing the compounds and information obtained by Elion and co-workers[274-277] and by Hitchings and co-workers[278-281] on purines and pyrimidines in relationship to the biosynthesis of nucleic acid, Burchenal and co-workers[282-284] found that a purine antagonist, 6-mercaptopurine (Purinethol), caused complete or partial remission and clinical improvement in approximately half of the children with acute leukemia treated. Adults with acute leukemia[285] or afflicted with early and late chronic myelocytic leukemia

has been re-evaluated as a procedure to be used only in selected cases rather than indiscriminately. Results seem to justify the operation when the criteria of Ferrata and Fieschi[304] are followed with certain modifications. As indications, they included thrombocytopenia on the basis of hypersplenism, splenomegaly, and hemolytic anemia. The bone marrow in leukemia will not likely present as favorable a picture as desirable, but operation is contraindicated only if the numbers of nucleated red cells and megakaryocytes are reduced to a pronounced degree. In the case of hypersplenic thrombocytopenia and pancytopenia, the calculated risk of operative intervention in patients with leukemia may well be rewarding,[135, 305-310] with improvement lasting for a number of years. However, splenomegaly alone is not an indication for splenectomy, responding as it frequently does to chemotherapy, irradiation, or a combination of the two. When the great size of the spleen and associated pain are otherwise unmanageable, removal becomes justified. Dameshek and associates,[311] Wintrobe,[214] and others advise splenectomy only after failure of steroid therapy when hemolytic anemia is the reason for considering surgery. Better results are obtained if the spleen is removed early in the course of the disease. Occasionally, splenectomy is performed for the cytopenia before leukemia is recognized,[305] and the diagnosis should always be kept in mind in this group of cases. Thymectomy[312] has not been beneficial in clinical cases of lymphogenous leukemia.

## SUPPORTIVE THERAPY[313-315]

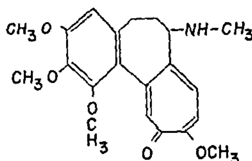
A forthright, positive, and cheerful attitude on the part of the physician assures the patient and his family that something can be done, and therapy should be directed toward prolonging the useful and happy period remaining before the inevitable outcome. In children, particularly, the periods of hospitalization should be reduced to a minimum. Leukopenia is not necessarily an indication for transfusion, and the hemoglobin value may return to normal in anemic patients without transfusion after appropriate therapy. For this reason, transfusions *should be deferred unless the necessity is obvious. If transfusions do* become necessary, they can be given on an out-patient basis. Also, prophylactic antibiotic therapy ordinarily is not advisable in the absence of a specific infectious process. The therapist should guard against the mistake of deferring definitive therapy too long with the idea that the patient may need therapy more urgently in the future. Treatment should begin when there are indications, since the patient's disease may become refractory to treatment, forfeiting palliation earlier in the disease.

## MANAGEMENT OF LEUKEMIA[161, 240, 316-328]

*Acute Leukemia.*—Both lymphoblastic and myeloblastic leukemia respond to antimetabolites and lymphoblastic leukemia responds to steroids. The meta-

*tion for this type of therapy, although the usual side effects* appear with doses of this magnitude. Ranney and Gellhorn[92] advise antacids and 3 Gm. potassium chloride daily as part of the prednisone and prednisolone therapy. Fessas and co-workers[295] confirmed the excellent results of treatment for acute lymphoblastic leukemia, but they advised caution in using cortisone and corticotropin in other forms of leukemia because response was not only less likely to occur but also the process seemed to be accelerated in some cases.

*Desacetylmethylcolchicine.*—Desacetylmethylcolchicine (Demecolcin, Colcemid), extracted from *Colchicum autumnale* by Santavy and Reichstein,[296] is thirty to forty times less toxic than colchicine in doses producing the same antimitotic effects[297] Following initial studies of Moeschlin and co-workers,[298] others have tested its therapeutic effects.[299-303] Remission of chronic granulocytic leukemia occurs frequently when this drug is administered, although relapse ensues rather promptly if it is discontinued. Leonard and Wilkinson[302]




Desacetylmethylcolchicine
(Colcemid, Demecolcin)

pointed out that the aplastic anemia, thrombocytopenia, and precipitation of acute myeloblastic leukemia, which are disadvantages of busulfan therapy, were not seen in a small group of cases they treated with Demecolcin. Initially, Demecolcin may be given in daily doses of 3 to 10 mg. by mouth. When the leukocyte count diminishes, a daily maintenance dose of 3 to 5 mg. is usually adequate with minimal side effects[299] A similar compound, N-desacetylthiocolchicine, has been found to have similar therapeutic effects by Huguenin and associates[303] and by Burchenal and Krakoff,[141] but additional data are desirable in order to evaluate the relative usefulness of this agent and Demecolcin in comparison to other types of therapy

SURGERY

Other than biopsy of soft tissues and bone marrow to establish the diagnosis, surgery is useful in leukemia only when splenectomy is indicated After the first splenectomy for leukemia by Bryant[105] in 1866, the procedure was tried extensively and abandoned as inadvisable. In recent decades, however, the operation

mine the exact usefulness of the colchicine group of compounds in the treatment of chronic myelogenous leukemia.

A tremendous amount of experience has been accumulated in the use of localized and total-body irradiation in chronic leukemia. A comparison of the results of irradiation treatment and chemotherapy is difficult, but there is no clear-cut superiority of chemotherapy to irradiation in the therapy of chronic leukemia. One type of treatment may be a useful supplement to the other. Irradiation of the spleen and localized lesions often is a useful adjunct to chemotherapy. The methods of continuous, titrated dosage of total-body and $P^{32}$ irradiation first described by Osgood[329] have produced results which are quite impressive.

In the treatment of the individual case of chronic lymphocytic and granulocytic leukemia, the therapist has at his command a comforting array of methods for definitive therapy (Table XXIV), as well as transfusions and antibiotics as supportive therapy for leukemia. Clearly, the possibility that additional chemotherapeutic agents may be more effective encourages laboratory and clinical screening programs, such as current investigations on the effect of antibiotics and biologic filtrates alone and supplemented with irradiation.[330, 331] In addition, much is to be learned about the most effective combinations and sequences for using the agents at hand

bolic antagonists of folic acid, purines, and glutamine are useful chiefly in children and more rarely in adults. Fortunately, resistance to one does not imply resistance to the remainder, and one agent can be used as supplemental therapy to the other. Serial treatment, therefore, may offer more than the use of one of the antimetabolites alone. Response to the antimetabolites requires a longer period of time, but remissions are longer in comparison to the results of steroid therapy. In acute leukemia, therefore, the folic acid, purine, and glutamine antagonists should be reserved for those cases in which waiting for a response for therapy is justified On the other hand, steroids exert their effects rapidly but the response is not prolonged. Therefore, in any patient who is acutely ill, particularly with hemorrhagic diathesis, steroids should be the initial form of therapy. In children, Amethopterin and mercaptopurine are the preferable drugs to use, whereas mercaptopurine probably is more advantageous in adults.

*Chronic Leukemia.*—A wider choice of agents is available for the treatment of chronic granulocytic and lymphocytic leukemia in comparison to effective measures for the acute disease. Benzene and arsenic are no longer commonly used, and urethan is used much less frequently than formerly. The alkylating agents, nitrogen mustard, triethylene melamine, and THIOTEPA, may cause regressions in both granulocytic and lymphocytic disease, but probably should be reserved as supplemental therapy with triethylene melamine being the preferred treatment. Superior to these agents for granulocytic leukemia is busulfan, and discontinuous therapy is practically free from undesirable secondary effects Chronic lymphocytic leukemia responds to chlorambucil, although somewhat inconstantly This, however, represents the most nearly specific of all the therapeutic agents for treatment of lymphogenous disease. In chronic granulocytic leukemia, the purine antagonists also may be effective occasionally. Of these, 6-mercaptopurine probably is the drug of choice, since neither thioguanine nor chloropurine offers additional advantages Responses also occur following administration of Colcemid, but sufficient experience has not accumulated to deter-

TABLE XXIV

RESPONSE OF LEUKEMIA TO THERAPY

| | ACUTE | CHRONIC | |
|---|---|---|---|
| | | MYELOCYTIC | LYMPHOCYTIC |
| Irradiation | – | + | + |
| Chemotherapy | | | |
| Arsenic | – | + | – |
| Urethan | – | + | ± |
| Mechlorethamine | – | + | + |
| Triethylene melamine | – | + | + |
| THIOTEPA | – | + | + |
| Busulfan | – | + | ± |
| Chlorambucil | – | ± | + |
| Folic acid antagonists | + | ± | ± |
| Purine antagonists | + | ± | – |
| Steroids | + | ± | ± |
| Demecolcin | – | + | – |

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